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PHYSIOLOGY AND PATHOLOGY OF THE KIDNEY
Scientific Editor . . . CLIFFORD WILSON

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THE LIVER: SOME PHYSIOLOGICAL AND CLINICAL ASPECTS
Scientific Editor . . . . N. F. MACLAGAN

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AUTONOMIC NERVOUS SYSTEM
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Physiology and Pathology of the Kidney: Introduction

This is a symposium on the physiology and pathology of the kidney, written by clinicians and others in close touch with clinical departments. It does not of course pretend to be exhaustive but is a sample of work which is in progress at the present time. There are several reasons why it is not written by physiologists: in the first instance the members of the Planning Committee, being clinicians, are much more closely in touch with those workers whose interest in the kidney has originally come from the patient. They therefore take the responsibility for this bias. Secondly, the physiologists have had a recent forum for the presentation of their work in the Cushny Memorial Lectures delivered in 1955 and recently published[1]. Thirdly, the exciting progress of medicine over the last 25 to 30 years largely arises from the application of physiology to clinical medicine. Nature poses certain problems such as renal oedema, aminoaciduria and chronic renal failure which are difficult, if not impossible, to reproduce in the experimental animal, and which must therefore be studied in man.

Three outstanding discoveries have influenced recent work on the kidney very considerably. I refer to the flame photometer which has facilitated so greatly the study of electrolyte metabolism;

[1] Reviewed on p. 73 of this number of the Bulletin.—Ed.

chromatography, which has made possible the study of specific failures in the tubular reabsorption of amino acids[1]; and the discovery of aldosterone which now influences all our thinking on the relation of the kidney to sodium and potassium. The discovery of aldosterone-producing tumours has been a further stimulus to clinico-physiological interest.

These discoveries have been partially responsible for a general change of emphasis in current interest, a change from tests of renal efficiency (such as glomerular filtration rate and renal plasma flow) to the study of the metabolic effects of renal disorder—a study which starts from the concept of the kidney as a homeostatic rather than an excretory organ.

I am very much indebted to all those who have contributed to this volume, and amongst their articles the reader will find many examples of the trends to which I have just referred, but in addition he will find examples of the exploitation of new techniques, radiological, histological, and histochemical, and of the use of radioactive materials in the elucidation of physiological problems. The relation of the kidney to hypertension, which seemed so near to solution after Goldblatt's experiments were published in 1934, is still not fully solved, and the adverse effect of severe hypertension on renal function is a matter closely concerning the clinician.

The recent work of McCance and Widdowson sees the kidney more from the view-point of the biologist, and Kennedy finds lesions as a result of over-nutrition which appear to represent premature senility and are similar to those which we have produced by partial nephrectomy, all these circumstances seeming to lead first to hyperplasia and then to degeneration. McCance's work, and also that of Black, raises questions not only of "how" and "what" but of "why". It is strange that, in a scientific world which recognizes evolution, teleology has been so long eschewed.

The field of renal pathology and physiology illustrates very well a process which is going on in all branches of medicine. The application of science to clinical problems and the increased complexity of knowledge necessitate more and more specialization so that no one man today would claim to be a competent authority on all branches of renal function and disorder or on all methods of investigation of these problems. Yet the worker whose interest starts in renal disease finds himself less isolated from workers in other fields than ever in history.

I am not greatly attracted to diagrams with arrows leading in different directions, but I confess that there is some temptation to put one in just here. But let us use the English language instead. He who starts the serious study of renal disease will soon find himself closely concerned with physiology, pathology, and biochemistry. On the clinical side he will perforce become interested in the pituitary and adrenal glands, and (through hypertension) in cardiology and neurology. Disorders of metabolism of many kinds, especially problems concerning electrolytes, water and proteins, he will need to unravel; and some of his most interesting cases will come to him from the obstetric, orthopaedic and neurological departments, if not from the psychiatrist to whom patients with intermittent potassium depletion, or generalized bone pain, are usually sent. Fascinating examples of hereditary defects will divert his interests into genetics and biology, and the diagnosis of an obscure anaemia and even of mental deficiency may find its first clue in the examination of the urine. In treatment he will require the co-operation of the physiologist, the pharmacologist, the physicist and the surgeon.

Truly, the study of the kidney is not a narrow specialty.

Robert Platt

[1] See Chromatography (*Brit. med. Bull.* 1954, Vol. 10, No. 3).—ED.

We wish to thank Professor Robert Platt for having acted as Chairman of the committee that planned this number of the Bulletin, and for all the valuable advice and help he has given throughout.

NEW THOUGHTS ON RENAL FUNCTION IN THE EARLY DAYS OF LIFE

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1 The basis of comparison
2 Unconsidered homeostatic mechanisms
3 Growth and food
4 Conclusions
References

Until about 1940, few seem to have considered that the function of the kidney in the new-born animal was a matter for serious investigation. If people thought about it at all they probably argued that, since the internal environment was normally maintained in a stable state directly after birth as at all ages, the kidney must always function in a similar way. This was perfectly sound reasoning and it was supported by some (Levy, 1926) if not entirely by all the work which had been done up to that time (Aschenheim, 1919; Wengraf, 1921; Lasch, 1923) on the renal function of young children. McIntosh, Möller & Van Slyke (1929) and Cullen, Nelson & Holmes (1935) had shown that the urea clearances varied closely with the surface area of the body down to the age of about two. There seemed little reason to face the technical difficulties of trying to study renal function in the new-born animal.

In 1941, however, McCance & Young published a paper which showed that the urea clearances, and also the sodium (Na) and chloride (Cl) clearances, were lower in the first fortnight of life than they were in adults when the comparison was made on the basis of surface area. They showed also that, at small urine volumes equated on the same basis, there was always a lower concentration of osmolar material in the urine of the new-born than in that of the adult. This paper drew attention to the subject and it was followed by many others. The findings in infants were compared with those in adults although the investigators did not always make a special study of the latter themselves. The approach, however, was always, or nearly always, on the lines which had been used to work out the function of the kidney in later life. Most of the investigations were made after the administration of water, and, therefore, at high rates of urine flow. A great deal of this work has been reviewed by McCance (1948, 1950); Smith (1951); Heller (1951); Barnett & Vesterdal (1953); McCance & Widdowson (1952a, 1952b, 1954a).

1. The Basis of Comparison

Some rather instructive points arise out of the consideration of all this work. (i) For some aspects of renal function, the results obtained on adults and infants can be compared directly without reference to the weight of the kidney or the size of the body. This applies to the total osmolar concentration of the urine produced under comparable degrees of dehydration, and there would seem now to be no doubt that from plasmas of equal osmotic tonicity the new-born animal produces a much less concentrated urine than the adult. (ii) Functions which may be expected to vary with size, such as the glomerular filtration rates and the urea clearances, can be compared only if some basis can be found for eliminating the effects of size; and there has been a general desire to do so because the approach has always been from adult physiology, and the problem posed has been: in what way does this or that aspect of renal function in infancy differ from the same function in adult life? (iii) Most of the work on man has been carried out by medical personnel who have generally made their comparisons on the basis of surface area. The animal studies have mostly been made by physiologists and pharmacologists (Heller, 1951), and these workers have usually taken body-weight as the basis for their comparisons. Although McCance & Young (1941) had shown how different the results could be made to look by changing the basis of comparison, other investigators were not sufficiently interested in this to go into the implications, which were not generally realized or critically examined.

McCance & Widdowson (1952a, 1952b) again made a study of the best basis of comparison to use, and came to the conclusion that the total body-water was probably the one to choose, at any rate for functions which depended mainly upon the glomerular filtration rate, and for the excretion of water. They reserved judgement on the most satisfactory basis for the electrolytes; for these Friis Hansen (see Vesterdal, 1955) has given reasons for preferring the volume of the extracellular fluids (ECF). He considers that, during growth, surface area and the volume of these fluids vary together, and that the success with which surface area has been used to eliminate the effects of size in renal work is due to this. A plea for considering the ECF was put forward so long ago as 1944 by Newman, Bordley & Winternitz, and the subject has been discussed again recently by Ikkos (1956). These authors were concerned only with adults.

A new-born baby weighs only one-twentieth as much as a man, but his surface area is one-eighth as great. If surface area is taken as the basis of comparison, therefore, the kidney appears to be less efficient than if the comparison is made on the basis of body-weight or body-water. All bases of comparison, however, indicate that the glomerular filtration rate in the newly born is very much lower than in adults. In man, comparison on the basis of body-weight or body-water suggests that, in early childhood, the filtration rates rise well above those of adults, whereas there would have been no indication of this if surface area had been used.

If the object of the investigation has been to compare the relative efficiencies of infantile and adult kidneys, it is impossible to avoid drawing conclusions from the results, but clearly this must be done with great caution and always with clear reference to the basis used. The real object, after all, at each age is the study of homeostasis, i.e., to ascertain the magnitude and the efficiency of the ways and means at the disposal of the body for maintaining the stability of its internal fluid compartments under the normal and abnormal stresses to which they may be subjected, and not to compare isolated renal functions of infants with those of adults or older children of a different

size. There is, therefore, much to be said for studying each age and size as a problem in itself, and not always trying to compare one age or size with another. The attempts to make such comparisons have tended to blind us to much of the interesting and important physiology of infancy, as the remainder of this article will set out to show.

2. Unconsidered Homeostatic Mechanisms

As already stated, whether the basis of comparison be surface area or body-weight, the glomerular filtration rates and clearances which depend upon them, and also the Na and Cl clearances, always look much lower in the first few days of life than after maturity has been attained. If these conclusions are to be taken at their face value, it is difficult to understand how and why the body-fluids of young animals contain concentrations of urea, Na and Cl that would be considered normal in adult life. The new-born rat, for example, has a very low urea clearance if compared with an adult on the basis of body-weight and it would be almost negligible on the basis of surface area; yet the blood urea is no higher than that of an adult after the first day or two of life (McCance & Otley, 1951). The low Cl clearance in human infancy has always been regarded as well-nigh pathological, certainly as a weak point about the infant's homeostatic mechanisms, and the recognition of this has been a valuable help in therapy; but no one has examined the matter from the truly physiological point of view. What for example is the significance to the infant of these low clearances? How is homeostasis maintained? Is there any connexion between the low NaCl clearance and the large volume of ECF present in the body at this age? Considerations such as these indicated that there was something incomplete about our ideas of homeostasis in early life, and perhaps about current conceptions of the kidney as the centre-piece in the control of the stability of the internal environment.

A new line of thought was opened up by experiments of McCance & Strangeways (1954). These authors were studying the renal function and general metabolism of normal full-term infants under conditions of starvation and water restriction. They found that these infants derived only some 4% of their basal calorie requirements from protein breakdown, whereas adults who had been subjected to a similar degree of starvation and hydropenia relied upon their tissue proteins for some 17–18% of their basal calories. The results of these experiments were not at once applied to the study of the homeostatic mechanisms of the new-born, but it is evident that, had the infants derived as great a percentage of their basal calorie requirements as adults from breaking down their tissue proteins, the concentration of urea in their body-fluids would have gone up much higher than it in fact did. This was brought home to us by a study of some infants who had been born only after a prolonged and difficult labour (McCance & Widdowson, 1954b). In some cases this much increased the breakdown of tissue protein without greatly affecting the urea clearances, and the concentration of urea in the serum rose to high levels.

Some very abnormal serum urea values have been found by investigators from time to time in the new-born infant. Snelling (1943), for example, described several babies aged from one to four days who had been born after prolonged and difficult labours, and were found on admission to hospital to have high values for the non-protein nitrogen in the serum. Jonsson (1951) published a description of three infants who were, in his opinion, suffering from lower nephron nephrosis following asphyxia neonatorum. His findings make this rather an unsatisfactory diagnosis (see McCance & Widdowson, 1954b), and it is more reasonable to attribute the high blood urea to abnormal protein breakdown. Campbell & Dales (1952) described a baby with sclerema neonatorum who was found to have a blood urea of 900 mg./100 ml. on the seventh day of life, and histologically normal kidneys after his death on the eighth day. These authors went into the question of how to account for this high figure even with completely functionless kidneys, but did not consider abnormal tissue destruction. This may have been the explanation, but there is another possible one.

3. Growth and Food

If an animal is growing, some of the material in its food is incorporated into the structure of its tissues and does not, therefore, present itself for excretion by the kidney. We had always appreciated that this must relieve the kidney of the new-born animal of some of the work which it was called upon to do in adult life, and therefore help the immature kidney of the new-born toward maintaining the blood urea within normal limits. The possibilities of this in the new-born infant, however, were made clearer to us by the discovery that the starving new-born infant broke down so much less tissue protein than a starving adult. The human infant moreover grows relatively slowly. A fast-growing animal which was being adequately fed might be expected to show up the effects of growth even more clearly. The pig was chosen to prove or disprove this (McCance & Widdowson, 1956). Three piglets of about the same weight were selected from each of four litters at birth and fed every two hours for about forty hours by a stomach tube. Their urine was collected quantitatively in a metabolism cage. One of the three piglets was given sow's milk, a second the same volume of a mixture of evaporated cow's milk and pig serum, the third nothing but an equal volume of water. There was little to choose between the level of urea in the blood brought about by these régimes. The reasons for this were:

1. Although the protein in the food provided 3,500 mg. of amino-acid N/kg./day, 90% of this was incorporated into tissue protein and only 10% of it appeared in the body-fluids as urea, and required to be excreted if the concentration in the body-fluids was to remain the same. Table I shows the nitrogen balances of piglets which were given milk or water, and

TABLE I. THE NITROGEN BALANCES OF NEW-BORN PIGLETS GIVEN MILK OR WATER

	Animals given milk or milk mixtures	Animals given water
N intake (mg./kg./24 hr.)	3,500	0
N output (mg./kg./24 hr.)	350	250
N balance (mg./kg./24 hr.)	+3,150	−250

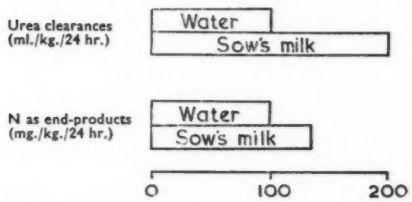
the capacity of these animals to grow when they were given a satisfactory diet is clearly shown by the colossal positive balances which were obtained. Much more of the protein nitrogen in the food would undoubtedly have appeared as urea if the food had not contained a satisfactory mixture of amino acids

and almost all of it—or at any rate an equivalent amount—would have appeared as urea in the body-fluids of an adult. This emphasized the extreme importance of growth in the regulation of the stability of the internal environment and also how desirable it is to have a satisfactory mixture of amino acids in the diet of a new-born animal. Some dislocation between appetite and growth was probably the reason why the baby studied by Campbell & Dales (1952) had a blood urea of 900 mg. on the seventh day of its life in spite of perfectly normal kidneys. It was certainly the cause of the "uraemia" which Schultze and his co-workers reported in new-born rats (Schultze, 1949; Schultze & Halvorson, 1949). If, moreover, the capacity of an infant for growth is absent or has failed, feeding by stomach tube could raise the level of urea in the body-fluids to very high levels—and may very well have done so without the real reason being recognized.

2. Some ingredient in the piglets' food, almost certainly the protein, led to the hypertrophy of their kidneys, and to a small increase in the urea clearances and excretion of urea nitrogen. These effects were definite, but small, considering the relatively enormous amount of protein nitrogen in the food. They were, however, enough to enable the body to excrete the nitrogenous end-products of the small percentage of the dietary protein which underwent catabolism (fig. 1), and homeostasis was maintained.

FIG. 1. THE EFFECT OF FOOD ON THE UREA CLEARANCE, AND ON NITROGEN APPEARING AS END-PRODUCTS OF PROTEIN METABOLISM

(values on water taken as 100)



The food contained 3,500 mg. of protein N/kg./24 hr.

The human infant has a much lower Na and Cl clearance than an adult, and, if there is too high a concentration of these mineral elements in the fluid food administered, their retention follows, and high values of Na and Cl may be found in the serum. The mixture of evaporated cow's milk and pig serum contained about 0.5% of NaCl, which was a higher concentration than that in sow's milk, and the responses of the piglets to which the mixture was given may be summarized in the following way. (i) The concentration of Na and Cl in the serum rose to an abnormal level. (ii) The piglets retained far more Na and Cl than could be accommodated in the ECF without a considerable expansion in its volume. (iii) This expansion in volume took place—as shown by a large gain in weight (Table II). These animals, therefore, had been forced into a state of hypernatraemia coupled with an expansion of the extracellular volume. This syndrome must be uncommon and difficult to produce in a healthy adult animal, although something of the kind seems to have been met with in hospital patients (Black, 1952). It would almost certainly be corrected by a normal man in his usual environment by the stimulus of thirst calling for further expansion of the ECF until its

TABLE II. THE SODIUM BALANCES AND SERUM LEVELS AND THE GAINS IN WEIGHT OF PIGLETS GIVEN SOW'S MILK OR AN EQUICALORIFIC MIXTURE OF EVAPORATED COW'S MILK AND PIG'S SERUM

	Animals given sow's milk	Animals given evaporated milk mixture
Na balance (mg./kg./24 hr.)	+163	+442
Serum Na (m-equiv./l.)	149	171
Gain in weight (g./kg./24 hr.)	+89	+177

osmolar concentrations became normal. The restoration of its volume to normal would follow. By a small reduction in the salt intake of these piglets it is likely that this abnormality in the internal environment could have been maintained indefinitely as a "steady state", probably to the great disadvantage of the growing organism. We had some experience with what I believe to have been this syndrome some years ago (see also Aldridge, 1941) when we were attempting to feed premature infants on amino-acid mixtures that had been made by hydrolysis of the parent protein with hydrochloric acid followed by neutralization with soda. Correction did not take long once the salt intake had been reduced, but the steps in the process were not investigated. The syndrome is not at present a well-recognized one; it is not discussed by either Finberg & Harrison (1955) or by Weil & Wallace (1956). It may be a new one, and its genesis will certainly have to be further explored. Its origin is likely to turn out to be part and parcel of the mechanism underlying the large extracellular volume in infancy, but at present an excess of aldosterone in the circulation does not appear to be the cause, for in adults, at any rate, such an excess generally presents as a potassium deficiency (Conn, 1955; *British Medical Journal*, 1956).

These experiments may turn out to be of importance in connexion with the interesting new clinical syndrome of persistent hyperelectrolytaemia in infants, coupled with diabetes insipidus and a failure of the kidney to respond to posterior pituitary hormone. This was first described by Waring, Kajdi & Tappan (1945), and accounts of the literature up to about a year ago have been given by Luder & Burnett (1954); Kirman, Black, Wilkinson & Evans (1956); and particularly by Macdonald (1955). The babies get dehydrated, and their primary lesion is almost certainly a failure to reabsorb water, but when their serum Na and Cl are normal they appear to reabsorb a very high proportion of the Na in their glomerular filtrates. An account of this syndrome was given by Dr Winifred Young at the 27th Meeting of the British Paediatric Association (Young, Levin & Russell, 1956), and of the successful way in which these children's internal environments could be maintained within normal limits by low-electrolyte diets, and the infants thus given an entirely new lease of life.

Whether or not there is any fundamental connexion between our syndrome of hypertonic oedema and the new clinical syndrome, it is quite clear that the composition of the food and the ability to grow are prime factors in regulating the stability of the internal environment in the new-born animal. They are factors seldom taken into account, but they are extremely important ones, particularly in fast-growing animals

like the rat, which appear to have such rudimentary renal function at, and soon after, birth. They should always be in our minds when we accept the responsibility of giving the new-born child or animal a food which it will not accept by the usual channels, or which has not been provided for it by nature.

4. Conclusions

If the object of the investigation is to compare the magnitude of certain functions of the infant's kidney with that of the same functions in adults, it is essential to make use of some basis of comparison which will eliminate size. Several have been used, and the conclusions drawn from the investigation may differ according to the basis employed. The results should always be considered in the light of the general function of homeostasis.

In fast-growing animals like the pig and rat, the balance between anabolism and catabolism may be more important than the kidney in maintaining homeostasis in the new-born. The relative importance of these mechanisms varies with the rate of growth, and in slow-growing animals such as man the kidney is relatively more important—and upon test shows itself to be more highly developed—than it is in the rat.

Anyone who would understand the function of the kidney in infancy must think of it in terms of the physiology of the whole animal. We shall only deceive ourselves further if we continue to compare functions isolated in a narrow way, and on some entirely artificial basis, with functions similarly isolated in adults.

It is possible to take such a broad view only by integrating the work and thought of many investigators, none of whom were in possession of enough facts to make the integration. Many of these have not been quoted in this article: much of their work may be unknown to us; to all of them we extend our thanks.

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TUBULAR SECRETION OF POTASSIUM

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1 Evidence for tubular secretion of potassium
  a Clearance of potassium higher than glomerular filtration rate
  b Analysis of tubule fluid
  c Relationships between potassium and hydrion excretion
  d Observations with ^{42}K
2 Discussion: potassium secretion in homeostasis
References

The germ of some recent advances in our knowledge of renal function can be discerned over a century ago in the views of Bowman (1842). While he noted how apt the glomerular apparatus was for a filtration process, he thought that this applied to water, and perhaps also to foreign substances, and that the normal urinary solids were later added by tubular secretion. Later work has established beyond reasonable doubt that the glomerular filtrate contains the solids of plasma except for proteins and lipids; and that it is formed in amounts much greater than the final volume of urine. These facts make it plain that the quantitatively preponderant activity of the tubules is that of reabsorption. The formulation of the " modern " filtration-reabsorption theory by Cushny (1917) is a landmark of renal physiology, which pointed the way to the studies of Homer Smith on the measurement of glomerular filtration rate (GFR) (Smith, Goldring & Chasis, 1938), and of Richards on tubular micropuncture (Richards & Walker, 1936)—studies which are now the chief support of the theory which presumably inspired them. Cushny's other concept of an " ideal reabsorbate " of constant composition is now untenable; quantitative observations on different urinary constituents have established that the kidney handles these separately, or at most in small related groups, so that the composition of the reabsorbed fluid is variable in response to the varied need for conserving or excreting specific substances. Moreover, for certain substances we must add to filtration and reabsorption the possibility of tubular secretion, giving a " three-component system of renal excretion " (Barclay, Cooke & Kenney, 1947). The first clear demonstration that tubular secretion was not a prerogative limited to aglomerular fish was the observation that substances such as phenol red, diodone and *p*-aminohippurate are excreted in amounts considerably exceeding the amount filtered, as inferred from measured plasma level and GFR. The question to be considered here is whether tubular secretion, so acutely foreseen by William Bowman, is a mere evolutionary survival, convenient for measuring renal plasma flow; or whether it is part of the kidney's daily business of regulating the composition of body-fluid.

1. Evidence for Tubular Secretion of Potassium

We have chosen potassium (K) as an example of a physiological substance which we believe to be eliminated by tubular secretion. It may be well to outline the evidence for this belief before considering the relevant studies in more detail:

i. Though excreted K is normally only a fraction of filtered K, it has been found in a number of circumstances, including renal disease, that excreted K exceeds the estimate of filtered K.

ii. Direct examination of tubular fluid suggests that, in the proximal tubule, reabsorption of filtered K may be virtually complete.

iii. Relationships between K and hydrion excretion are most easily explained by an ion-exchange secretory mechanism common to both.

iv. For some time after injection of radioactive potassium (^{42}K), the arterial K can be distinguished from renal-venous K; the K in the urine is then demonstrably different from arterial K, and akin to renal-venous K, which suggests that they have a common source in renal tissue.

a. Clearance of Potassium Higher than Glomerular Filtration Rate

The clearance of K (C_K) is the excretion of K per minute (E_K), divided by the plasma level ([K]); hence $E_K = C_K \times [K]$. The filtered load of K per minute is [K] multiplied by the GFR per minute, usually equated with inulin clearance (C_{IN}); hence filtered load (F_K) = $[K] \times C_{IN}$. Dividing one of these expressions by the other, the ratio of excreted to filtered K (E_K/F_K) is equal to the ratio of K clearance to inulin clearance (C_K/C_{IN}). If this clearance ratio is higher than 1, excreted K is higher than the estimate of filtered K; if the measure of GFR used is valid, this must imply tubular secretion of K.

A C_K/C_{IN} ratio in excess of unity was first observed by McCance & Widdowson (1937) in a patient with dehydration and alkalosis. Leaf & Camara (1949) found C_K higher than $C_{creatinine}$ in patients with renal failure; and Platt (1950) confirmed this finding, and added the observation of C_K/C_{IN} ratios > 1. All these observations were made on patients with a low GFR, so it remained possible that K secretion was an emergency mechanism invoked to compensate for grossly diminished filtration of K. However, Franglen, McGarry & Spencer (1953) have now shown that C_K/C_{IN} ratios > 1 can be induced at normal levels of GFR by combining oral potassium loading with hyperventilation alkalosis in normal human subjects. These observations show conclusively that secretion of K is a mechanism available to the kidney under conditions of stress; but they do not allow us to distinguish the proportions of urine K which are derived from filtered and from secreted K.

b. Analysis of Tubule Fluid

The results of Bott (1954) show that samples of tubular fluid derived from the lower end of the proximal tubule contain K in much lower concentration than in glomerular filtrate, which has essentially the K concentration of plasma. In *Necturus* at least, there seems to be a progressive fall in K concentration in samples obtained progressively lower down the proximal tubules, and in the two lowest specimens the K

concentration was only 20% of plasma K. The K concentration in the final specimen of urine is again very much higher, but much of this increase is no doubt related to distal tubular reabsorption of water without K. However, the ratio of K concentration to inulin concentration was also much higher in the final urine than in fluid from the end of the proximal tubule, which suggests that some K had actually been added in the distal tubule. Somewhat similar findings in the mammalian kidney have since been published by Wirz & Bott (1954). This method may well prove to be the most direct and convincing way of assessing K secretion; but at present the reported results are preliminary and incomplete.

c. Relationships between Potassium and Hydrion Excretion

It is now generally agreed that the formation of acid urine from alkaline glomerular filtrate is accomplished by the tubular secretion of hydrion in exchange for reabsorbed sodium (Na) (Pitts, 1950). Berliner, Kennedy & Orloff (1951) made the important observation that, when the availability of hydrion for secretion is decreased by a potent inhibitor of carbonic anhydrase, the output of K rises; this increased output of K can be inhibited by mercurials, suggesting that it is the result of an active secretory process. On the basis of this work, they suggest that much, if not all, of the normal excretion of K may be accomplished by ion exchange for Na, i.e. by tubular secretion. Mudge, Ames, Foulks & Gilman (1950) had already noted that mercurials decreased the high K excretion after K loading, but increased the low K output associated with water diuresis. This led them to formulate the cautious hypothesis, accepted by Berliner *et al.*, that " · · · the possibility is not remote that under normal circumstances a major portion of the K in the glomerular filtrate is destined for reabsorption and that the amount of K excreted in the urine is largely defined by the magnitude of the secretory process ".

Both these observations were made on dogs under the influence of potent diuretics; so it is of some interest that Black & Mills (1954), in a study of nocturnal electrolyte excretion in man after small doses of sodium and potassium chloride and bicarbonate, found that the observed changes were consistent with the Berliner hypothesis of exchange of (K + hydrion) for Na. They concluded that " renal adjustment to small electrolyte loads is accomplished by variations in the amount and character of cation exchange "—a process which involves tubular secretion of K. Likewise, Mills & Stanbury (1954) have presented evidence that in the diurnal rhythm of electrolyte output there are converse changes in the output of K and hydrion, the change in K being probably primary.

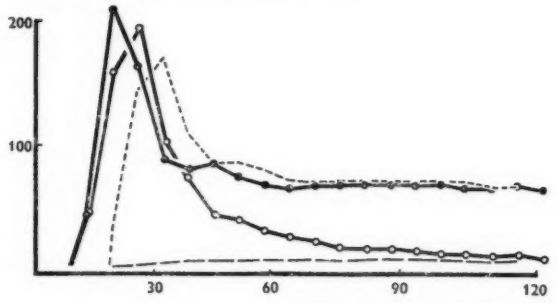
d. Observations with ^{42}K

Another way of studying the excretion of K in man under virtually physiological conditions has become available with the use of ^{42}K. The method depends in principle on the observation that, for some time after an intravenous injection of ^{42}K, the K in arterial plasma is of different specific activity from the K of renal-venous plasma. This makes it possible to distinguish between K derived from the plasma entering the kidney and K already present in the renal tissue; and so to assess in which category urine K falls.

We have assumed that renal-venous plasma K is representative of contemporary renal-tissue K; or in other words, there is complete exchange of plasma K during transit through the kidney, so that renal-venous K contains only a negligible amount of the K which has just entered the kidney. Many observations on animals, reviewed by Wilde (1955), have shown clearly that the rate at which K leaves the plasma is very high; during a single circulatory transit, replacement of entering plasma K by K derived from the tissue is practically complete. In order to assess whether this was true of the human kidney, we have examined serial samples taken over periods of a few seconds from the brachial artery and renal vein immediately following injection of 80 μc of ^{42}K and 15 mg. of T-1824 (Evans' blue) into an arm vein. The results of one such experiment are shown in fig. 1. The concentrations of ^{42}K and T-1824 are expressed as a percentage of the administered dose per litre of plasma, so that the curves for the two substances can be compared directly. It is apparent from the T-1824 curves that, while there is some delay in the passage of the injection-mass through the renal vein, as compared with the arterial passage, there is no substantial difference in the total amount of T-1824 which passes in the relevant time. On the other hand, only 4% of the injected ^{42}K found in the arterial plasma passes through the kidney on first circulation; and even this small amount may be an over-estimate, since some of the 4% of ^{42}K may already have left the circulation and returned to it. There is not, of course, any substantial net loss of K from the plasma during renal transit; the entering K has been replaced by K derived from renal tissue. We conclude from this evidence, and from analogy with the relevant animal experiments, that renal-venous plasma K is almost entirely derived from K already present in renal tissue, and not appreciably from the K which entered the kidney at the time of injection. Immediately after ^{42}K injection, the kidney tissue takes up a proportion of the injected dose which is related to the high renal blood flow, so that renal K contains ^{42}K in substantial amount, and so has a high specific activity.[1]

[1] The specific activity is the ratio of ^{42}K to total chemical K. We have expressed ^{42}K as a percentage of the residual dose—i.e., of the amount injected less the cumulative amount excreted—and chemical K as m-equiv. The specific activity is thus expressed as percentage dose/m-equiv. K.

FIG. 1. TIME-COURSE OF CONCENTRATION OF ^{42}K AND OF T-1824 IN BRACHIAL-ARTERY AND RENAL-VENOUS PLASMA AFTER INTRAVENOUS INJECTION OF ^{42}K AND T-1824



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Abscissae: time (sec.)

Ordinates: activity (expressed as percentage of administered dose per litre of plasma)

●——● T-1824 in brachial artery

- - - - - - - - T-1824 in renal vein

o——o ^{42}K in brachial artery

— — — — ^{42}K in renal vein

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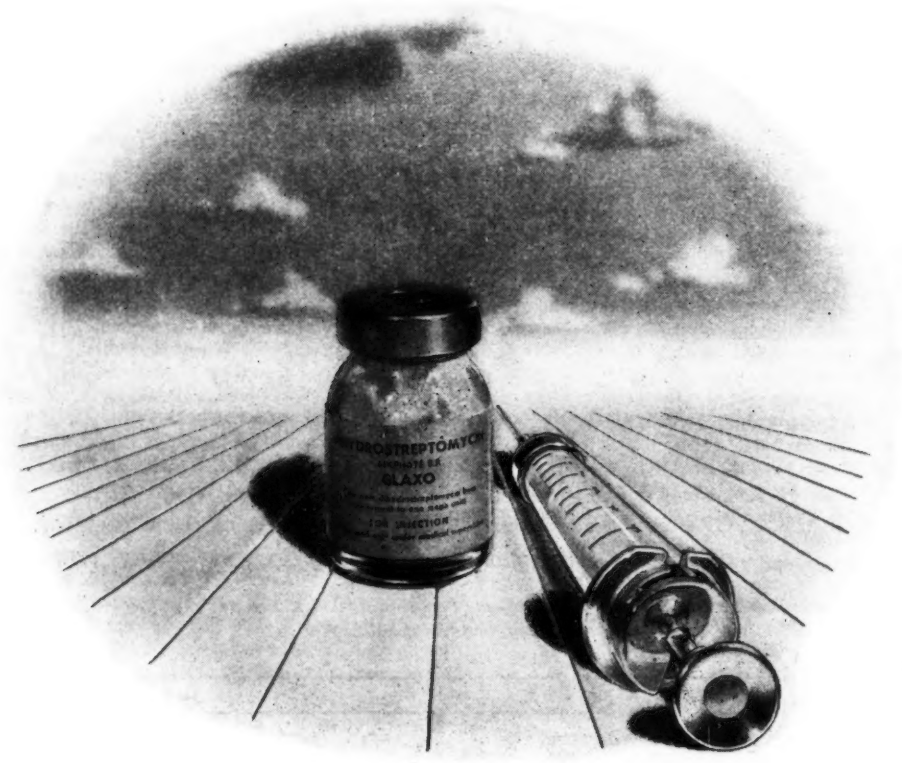

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Meanwhile a similar process has been going on in all other tissues, so that the ^{42}K quickly leaves the circulating plasma, and the specific activity of plasma K falls rapidly (Black, Davies & Emery, 1955). A minute or two after injection, the specific activity of arterial plasma K will have fallen below the level of specific activity established in renal tissue K by the early rapid uptake of ^{42}K; and in the period from 2 minutes after injection to upwards of 30 minutes, the specific activity of renal-venous plasma K remains very appreciably higher than that of arterial plasma K. This corresponds to the discharge of the pool of ^{42}K established in the kidneys during the period of first circulation of the injected mass.

During this half-hour period we have the opportunity to compare the specific activity of urine K with that of arterial and of renal-venous plasma. Representative results are shown in fig. 2. The specific activity of the K of renal-venous plasma is consistently higher than that of arterial plasma K. The specific activity of urine K closely resembles that of renal-venous plasma K, and is different from that of arterial plasma K. The specific activity of urine K is in fact a little higher than that of renal-venous K, for which three possible explanations can be suggested, though we have no evidence to decide among them:

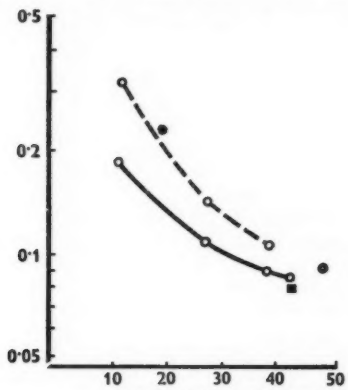
i. There is a mechanical delay in the appearance of urine in the bladder much greater than the time taken to draw samples from the renal vein. It may be that the samples from the two sources had the same specific activity at the moment of leaving the kidney, and that the observed difference merely reflects the amount by which the specific activity of renal tissue has fallen during the time taken for urine to reach the bladder.

ii. Secreted K is derived directly from renal cells, while plasma K is derived directly only from renal extracellular fluid. While the kidney is discharging ^{42}K, there may be a small gradient of specific activity between cells and extracellular fluid.

iii. The renal-venous K may be appreciably "contaminated" by traces of K which has just entered by the arterial plasma, and which is therefore of much lower specific activity. Urine K may then be a better sample of contemporary tissue K.

The main point, however, is that the K in the urine has essentially the character of renal-venous plasma K, which we believe to represent renal tissue K; and is appreciably different from the K entering the kidney by the artery, from which the K of glomerular filtrate is directly derived. There is no indication from our results that urine K is appreciably contaminated with filtered K; and they appear to support the proposition of Mudge *et al.* (1950) that urine K is substantially the result of tubular secretion, and contains little if any of the K filtered into Bowman's capsule.

FIG. 2. COMPARISON OF THE SPECIFIC ACTIVITY OF ARTERIAL PLASMA K, RENAL-VENOUS PLASMA K, URINE K, AND MIXED VENOUS PLASMA K



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Abscissae: time (min.)
Ordinates: specific activity (expressed as percentage of residual dose/m-equiv. K)

o———o arterial plasma K
o— — —o renal-venous plasma K
● ● urine K
■ mixed venous plasma K

2. Discussion: Potassium Secretion in Homeostasis

It is not surprising that tubule cells with a K concentration of about 100 m-equiv./l. should contribute K to a tubular fluid with a level of 5 m-equiv./l. or less over the greater part of the nephron; it is perhaps more difficult to see why filtered K should apparently make so little contribution to final urine K. But this is not the only way in which the kidney seems to court complexity, as if inspired by the motto: "Warum einfach machen, wenn man kann es komplizieren". Far be it from us to interpret the ways of the kidney, but it is perhaps worth noticing that an excretory mechanism based on secretion may be more adapted to the regulation of an intracellular cation than one based on filtration. There is evidence (Roberts, Randall, Sanders & Hood, 1955) that intracellular pH may be the immediate determinant of bicarbonate reabsorption, a process which is closely linked to hydrion secretion (Stanbury & Thomson, 1952); and, similarly, intracellular [K] could affect secretion of K, either directly, or through its influence on intracellular pH (Anderson & Mudge, 1955). Secretion of K, as we have seen, is often demonstrable at high rates of K output; but at low rates of K output, as in extrarenal depletion, there is no evidence to tell whether the remaining urine K, amounting to 1% of the filtered load or less, has been filtered or secreted. A failure to reabsorb 1% of filtered K would not be detectable by any of the techniques we have reviewed; so at present it must remain open whether K secretion is completely inhibited in K depletion. At any rate, it must be reduced to a very small fraction of its normal magnitude, let alone its maximal amount.

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THE VOLUME CONTROL OF BODY-FLUIDS

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University of Manchester

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2 Extracellular fluid
  a Renal handling of sodium
  b Adrenal cortex
  c Volume-receptor hypothesis
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3 Some clinical implications
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Some 60% of the human body consists of water, and the narrow limits between which, in health, body-weight fluctuates indicate the constancy of this fluid component. Osmotic pressures within the body-fluid compartments are predominantly determined by electrolytes. Since cations are fewer in number and more easily measured than anions (some of which are organic and highly labile) this review is to a large extent concerned with the metabolism of sodium (Na) and potassium (K), the predominant cations in extracellular and intracellular fluid respectively.

1. Intracellular Fluid

Calculation of intracellular osmolarity from known cation concentrations arrives at a figure some 40% higher than that of extracellular fluid (ECF). Even so, osmotic equilibrium between the two fluids has been generally assumed, and to explain the apparent discrepancy it has been suggested that a portion of intracellular cation exists in non-ionic form, or alternatively, that a large proportion of intracellular anions are polyvalent. Recently several workers have challenged this assumption. Opie (1956) has observed that kidney and liver slices swell in saline media unless the concentration is at least one-and-a-half times that of physiological saline. Robinson (1950) found that tissues in isotonic media do not swell as long as they respire normally, but that swelling develops when metabolism is inhibited by cold, cyanide, or oxygen lack. These workers believe that the cell normally maintains hypertonicity by the continual active extrusion of water.

However, Mudge (1951) and Leaf (1956) have demonstrated that the fluid entering the cells under these circumstances is not water alone but is isotonic with the medium. Leaf has pointed out that the swelling caused by metabolic inhibition can be explained by breakdown in the active extrusion of Na: intracellular colloid osmotic pressure then causes an entry of isotonic fluid from the medium and it is not necessary to postulate intracellular hyper-osmolarity.

Direct measurement of intracellular osmolarity has yielded conflicting results, apparently because any interference with cell metabolism causes a rapid increase in osmolarity from the breakdown of cell constituents. Perhaps, because of this difficulty, it is wisest to accept the lowest figures, those of Conway & McCormack (1953) who find cell osmolarity identical with that of ECF. Certainly in many circumstances the intracellular space as a whole behaves as if it were in osmotic equilibrium:

i. Potassium deficiency is accompanied by shrinking of the intracellular space (Black & Milne, 1952).

ii. Intravenous hypertonic saline causes a shift of water from the intracellular to the extracellular space (Hetherington, 1931).

iii. A large water load is evenly distributed over both spaces (Leaf, Chatillon, Wrong & Tuttle, 1954; Wynn, 1955).

2. Extracellular Fluid

The control of extracellular volume has recently received much attention, and the subject has been reviewed by Black (1954); Bull (1954); Robinson (1954); and Selkurt (1954).

Relative distribution of fluid between the intravascular and interstitial compartments is determined by the balance between, on the one hand, intracapillary hydrostatic and interstitial oncotic pressures tending to force fluid into the tissue spaces, and, on the other hand, plasma oncotic and interstitial hydrostatic pressures tending to force fluid into the capillaries. This hypothesis was first put forward by Starling (1896) sixty years ago and has recently been experimentally confirmed by Pappenheimer (1951). As interstitial hydrostatic and oncotic pressures are small and fairly constant, the relative amounts of intravascular and interstitial fluid depend on intercapillary pressures over the body and the total circulating plasma protein. The latter is in equilibrium with the general body-protein and returns to normal within a few days when either

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TUBULAR SECRETION OF POTASSIUM

Continued from page 9

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depleted by plasmapheresis or increased by plasma infusion (Whipple, 1956), but there is little information on how this constancy is brought about.

Extracellular osmotic pressure is carefully controlled by two delicate mechanisms: thirst, and the osmoreceptor-posterior pituitary mechanism described by Verney (1954). The "drinking centre" of Andersson & McCann (1955) and Verney's osmoreceptor are both situated in the hypothalamus but seem to be anatomically distinct. Increase in ECF tonicity stimulates thirst and the secretion of antidiuretic hormone, decreased tonicity abolishes these effects. Both mechanisms work rapidly and without the overswing of many biological adjustments; the water diuresis following ingestion of water, for example, is usually complete within two hours.

These osmo-regulatory mechanisms are unsuited to compensate for isotonic changes in ECF volume. The relative constancy of ECF volume indicates that the total amount of extracellular solute, and hence of Na, is closely regulated. As Na intake varies widely and extrarenal losses are small, this regulation is brought about by control of urinary Na excretion.

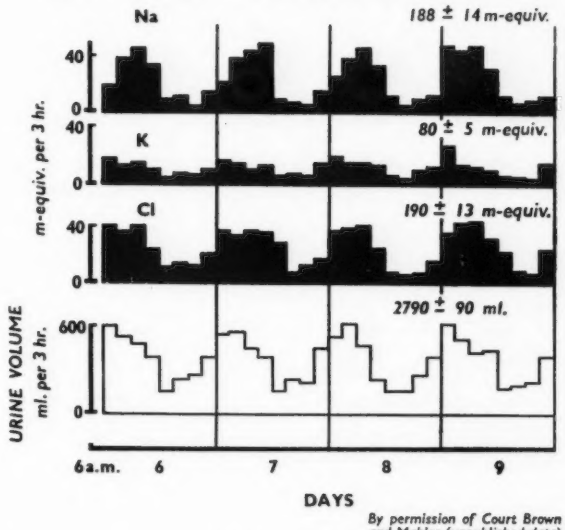
a. Renal Handling of Sodium

Measurements of the rate of Na excretion in the normal individual reveal large, apparently random, variations. Even on a diet of constant Na content Black (1953) has found daily variations in Na balance of the equivalent of as much as a litre of ECF. Important variables affecting excretion are exertion, emotion and posture, and when these are as vigorously controlled as possible a fairly constant pattern of Na excretion emerges after a few days. The results of such an unvarying regime, from unpublished data of Court Brown and Mahler, are shown in fig. 1. The chart shows not only a very constant Na excretion (the day-to-day fluctuations are only one-quarter of those encountered by Black) but also a wide diurnal rhythm in the excretion of the main electrolytes and water. The precise cause of this rhythm is still unknown.

A further difficulty which arises in the study of Na metabolism is the sluggishness of the renal response to sudden changes in intake. Borst (1954) has made use of carefully controlled observations such as those in fig. 1 to examine the effects of intravenous infusions of up to two litres of an artificial "extracellular fluid" (NaCl, 115 m-equiv./l.; $NaHCO_3$, 25 m-equiv./l.). The resulting Na diuresis developed slowly and took up to 48 hours for completion. In the opposite experiment—a very low Na intake—about 48 hours again elapsed before maximal reduction in Na excretion (Black, Platt & Stanbury, 1950; Rosenheim, 1951). But there is great individual variation in the rapidity with which Na balance can be attained, and Gamble (1951) reports one individual who took two weeks to reach equilibrium after an increase in intake of only 5 g. of NaCl daily.

Sodium is excreted by a process of glomerular filtration and tubular reabsorption. Approximately 600 g. of Na are filtered daily, but over 95% of this is reabsorbed in the renal tubules. Consequently an enormous range in Na excretion (over one thousandfold in the case of the healthy kidney) can be brought about by variations of under 5% in filtered or reabsorbed Na. Unfortunately renal clearance techniques are not sufficiently accurate to determine whether normal variations in Na excretion are caused by alterations in filtration or in tubular absorption. In the past, changes in filtration rate were thought to be the more important, but this view was based largely upon experiments on dogs, a species with a very labile filtration rate. In man the filtration rate is fairly constant and is not increased during the Na diuresis which follows isotonic saline infusions (Crawford & Ludemann, 1951; Green, Bridges, Johnson, Lehman, Gray & Field, 1950). Reduced tubular reabsorption must explain the Na diuresis under these circumstances. Black *et al.* (1950) have shown that an increase in tubular Na reabsorption takes place after a few days on a low-salt diet.

FIG. 1. EXCRETION RATES OF ELECTROLYTES AND WATER IN A PHLEGMATIC YOUNG MAN, AT COMPLETE BED-REST, WITH CONSTANT THREE-HOURLY FEEDS OF MILK AND BISCUITS



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Observations commenced on 6th day of regime. Figures on right are the means and standard deviations of the daily excretion rates.

Although the kidney has a rich nervous supply there is little evidence that tubular transport mechanisms are under direct nervous control. Renal denervation in the dog has, under some circumstances, led to an increase in Na excretion, but this is associated with a rise in filtration rate which is probably the result of altered vascular tone (Surtshin, Mueller & White, 1952). The most convincing evidence that the renal nerves do not play an important role in Na excretion comes from the experiments of Dempster & Joekes (1953) who showed that dogs in which the kidneys were transplanted (and therefore thoroughly denervated) maintained a normal ECF volume. However, there is considerable evidence that adrenal cortical activity profoundly affects tubular reabsorption of Na.

b. Adrenal Cortex

The adrenal cortex plays a role in the maintenance of ECF volume similar to that of the posterior pituitary in the control of ECF tonicity. Many of the manifestations of Addison's disease are due to shrinkage of the extracellular space following loss of Na in the urine. Sodium loss is not only to the exterior—there is in some cases an increase in intracellular Na which reverts to normal under the influence of cortisone (but not of deoxycorticosterone) (Wilson & Miller, 1953).

Aldosterone is much the most important mineralo-corticoid; it accounts for two-thirds of the Na-retaining activity of

adrenal-venous blood and is about 20 times as active as deoxycorticosterone in this respect. Normal subjects secrete such minute amounts that it has not yet been possible to discover whether it is responsible for normal variations in Na excretion; advance in this direction requires even more sensitive methods of assay than those at present available. Intravenous aldosterone has an action which lasts eight hours or more (Thorn, Sheppard, Morse, Reddy, Beigelman & Renold, 1955; Muller, Mach & Naegeli, 1955), and for this reason it seems unlikely that variations in aldosterone production could explain sudden changes in Na excretion following, for example, change from a standing to a lying position. Conversely the slow Na diuresis following intravenous saline could well be explained by a reduction in aldosterone secretion; the well-known differences in the timing of a water and a saline diuresis might be due to the difference between the time needed to inactivate intravenous antidiuretic hormone (one hour) (Klisiecki, Pickford, Rothschild & Verney, 1932) and aldosterone (eight hours).

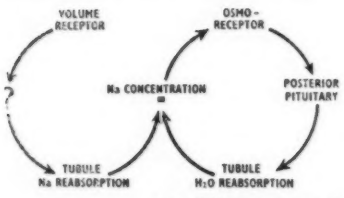
The stimulus for aldosterone secretion is still uncertain. The anterior pituitary does not appear to be necessary (Luetscher & Axelrad, 1954) and corticotrophin causes little or no increase in secretion (Cope & Garcia-Llaurado, 1954). Subjects on a low-salt diet (10 m-equiv. Na daily) excrete increased amounts (Luetscher & Johnson, 1954), but not unless K is provided (Laragh & Stoerk, 1955). Both Na and K would seem to be concerned in aldosterone regulation, but in the case of Na it does not seem to be the serum concentration that matters, rather the volume of some component of the ECF. Hyponatraemia is not necessarily present in sodium-depleted or oedematous subjects excreting increased amounts of aldosterone, and Farrell, Rosnagle & Rauschkolb (1956) have found that haemorrhage increases excretion without lowering the serum Na.

c. Volume-Receptor Hypothesis

Peters (1948) and Borst (1948) pointed out almost ten years ago that known mechanisms of body-fluid control could not explain the close regulation of ECF volume. They postulated the existence of a " volume receptor ", sensitive to changes in ECF volume or some closely related function, which regulates Na excretion by control of renal tubular activity. Such a mechanism would dovetail with that controlling extracellular tonicity in the manner shown in fig. 2 (from Bull, 1952).

Such a mechanism appears, to me, to be required to explain the constancy of ECF volume and the failure of changes in glomerular filtration to explain this constancy. There is strong evidence that the role of the question mark in fig. 2 is taken by the adrenal cortex. The chief problem remaining is the nature of the volume receptor itself.

FIG. 2. POSSIBLE MECHANISM FOR THE CONTROL OF EXTRACELLULAR FLUID VOLUME



Bull (1952) by permission of J. & A. Churchill

The circulating plasma is the most vital part of the ECF and the one most extensively investigated in this respect. Robinson (1954) has summarized the procedures which affect both the circulation and Na excretion. Generally speaking, Na excretion is increased by manoeuvres which increase the blood pressure or central circulatory volume: infusion of isotonic or hypotonic saline, lying down, compression of the legs by elastic bandages, or the closure of an established arteriovenous fistula (Epstein, Post & McDowell, 1953). Sodium excretion is decreased by procedures which diminish blood volume or cause pooling in the extremities: haemorrhage, passive standing, and obstruction of venous return from the limbs. Some of these last procedures—for example haemorrhage (Rydin & Verney, 1938)—also cause antidiuresis, probably through stimulation of the posterior pituitary.

Peters, Welt, Sims, Orloff & Needham (1950) and Cort (1954) have described a salt-losing state in certain forms of intracranial disease. This suggests that the volume receptor might lie within the skull. Experimentally, Viar, Oliver, Eisenberg, Lombardo, Willis & Harrison (1951) have found evidence for this: they inflated venous-occluding cuffs around the necks of seated subjects and found an increase in Na excretion. These results have not been confirmed by Bull (1954). Barbour & Bull (1954) have also reduced the cerebrospinal pressure by lumbar puncture without change in Na excretion.

The thorax has also received attention as the possible site of a volume receptor. Sims, Welt, Orloff & Needham (1950) have described a group of " pulmonary salt-wasters "—patients with various pulmonary lesions and symptomless hyponatraemia. Drury, Henry & Goodman (1947) and Sieker, Gauer & Henry (1954) used breathing under positive and negative pressure in a search for a receptor on the low-pressure side of the pulmonary circulation. No changes in Na excretion were observed, but breathing against a low pressure caused a water diuresis, and breathing against raised pressure, an antidiuresis. Henry, Gauer & Reeves (1956) have shown, in dogs, that this water diuresis is probably mediated by stretch receptors in the left atrium.

Bull (1954) has explored the possibility that the volume receptor is a chemo-receptor sensitive to changes in partial pressures of the respiratory gases. If this were so, breathing 10% oxygen or 5% carbon dioxide might be expected to reduce Na excretion. In neither case did this happen, but during CO_2 inhalation there was a moderate water diuresis.

The search for a volume receptor, or receptors, continues. It is worth noting that, if the efferent pathway of volume regulation is through the adrenal cortex, some of these experiments may have been too short in duration to influence Na excretion.

d. Volume Regulation versus Osmo-Regulation

The method of volume control envisaged in fig. 2 suggests that extracellular Na concentration and Na excretion do not necessarily proceed in the same direction. There are at least two interesting circumstances where they proceed in opposite directions, and, in a sense, volume regulation overrides osmo-regulation:

i. Black, McCance & Young (1944) found that individuals deprived of water retained Na even though the serum Na rose as a result of haemoconcentration. Elkinton & Taffel (1942) studied more severe dehydration in dogs and found that Na and Cl disappeared from the urine although

the serum Na rose by as much as 40 m-equiv./l. Large amounts of intracellular electrolyte (K and phosphate) appeared in the urine. The authors called this the " dehydration reaction " and interpreted it as an attempt to preserve ECF volume at the expense of intracellular water. More recent work of Bartter (1956) has shown that, in man, dehydration is accompanied by an increase in aldosterone excretion, which probably explains the urinary Na and K changes.

ii. Leaf, Bartter, Santos & Wrong (1953) investigated the effects of water retention induced by antidiuretic hormone (pitressin) in man. Water retention caused serum dilution, and falls in serum Na concentration to as low as 116 m-equiv./l.; at the same time there was a marked Na diuresis. Pitressin administration without water retention had no effect on Na excretion. These workers interpreted the sodium diuresis as a response to overexpansion of the body-fluid volume. It is probably mediated through the adrenal cortex; Muller, Riondel & Mach (1956) demonstrated a concomitant decrease in aldosterone excretion, and I found (Wrong, 1956) the expected delay of about eight hours between hydration and maximal Na diuresis.

Conflict between the interests of volume regulation and osmo-regulation was even better seen in the classic salt-depletion experiments of McCance (1936). Here progressive Na loss was at first accompanied by proportionate water loss, and ECF osmolarity was maintained at the expense of volume. When a Na deficit of about 350 m-equiv. was reached, water loss became less and further Na depletion caused hyponatraemia; volume was being preserved at the expense of tonicity. At this stage subjects lost their ability to have a water diuresis. Leaf & Mamby (1952) observed a similar failure of water diuresis in Na-depleted dogs, and demonstrated an increase in serum antidiuretic activity, probably of posterior pituitary origin. From these observations it seems that gross reduction of ECF volume stimulates the posterior pituitary, even in the presence of hypo-osmolarity, the usual inhibitor of the posterior pituitary.

3. Some Clinical Implications

a. Dehydration

Marriott (1947) pointed out that the clinical syndrome of dehydration, with subcutaneous desiccation, inelasticity of the skin, cold extremities and hypotension, indicates shrinkage of the extracellular space and hence loss of Na.

Paradoxically a pure water deficit does not, unless very severe, result in the clinical picture of dehydration: thirst, oliguria, and raised serum concentrations of Na and Cl are earlier signs. The thirst and posterior pituitary mechanisms are so efficient that, in temperate climates, a severe water deficit can develop only when one or both are damaged by disease:

i. In untreated diabetes insipidus, even when access to water is free, a considerable water deficit may develop with serum Na concentrations as high as 165 m-equiv./l.

ii. In any patient unconscious for a long period from any cause there is a danger of a water deficit. These patients cannot experience thirst, their attendants may have difficulty ensuring an adequate fluid intake, and water loss may be increased by fever or a complicating diabetes insipidus. The syndrome is not uncommon and cases have been reported by Allott (1939) and Higgins, Lewin, O'Brien & Taylor (1951). Unfortunately it is seldom recognized for what it is. Higgins *et al.* were impressed by the combination of hyperchloraemia and low urinary Cl—the dehydration reaction of Elkinton and Taffel—and interpreted the metabolic abnormality as a disturbance of Cl excretion.

b. Oedema

Most of the symptoms of congestive heart failure are due to retention of Na and expansion of the extracellular space. Although this may lead to further circulatory embarrassment it is probably mediated by the over-action of physiological mechanisms which normally maintain ECF volume, and is in the nature of a compensation for a failing circulation. The exact feature of circulatory failure which leads (by the hypothetical volume receptor) to Na retention is still unknown. In nephrotic and cirrhotic oedema, Na retention may arise by a similar mechanism, but here it is excessive loss of intravascular fluid into the tissues which stimulates Na retention. In all three forms of oedema there is a marked increase in aldosterone excretion (Luetscher & Johnson, 1954), and in nephrotic oedema this returns to normal if corticotrophin treatment results in a Na diuresis (Luetscher, Deming & Johnson, 1952). But excessive secretion of aldosterone alone cannot explain oedema in these diseases for, of the patients with proved aldosterone-secreting tumours, none has shown oedema, though they excrete even more aldosterone; moreover all have presented with K deficiency (Conn & Louis, 1956).

When depleted of Na, oedematous patients behave as normal subjects do. At first water is lost pari passu with Na; at a later stage water loss may not keep pace with Na loss, and hyponatraemia develops (the usual form of " low-salt syndrome "). These subjects lose their ability to have a water diuresis, and their serum contains increased amounts of an antidiuretic substance, probably of posterior pituitary origin (Leaf & Mamby, 1952). Laragh (1954) and Cort & Matthews (1954) have shown that some of these patients are K-depleted, with an increase in intracellular Na. The metabolic disturbance is complex; one of the possible causes of K deficiency is the increased secretion of aldosterone, the " secondary aldosteronism " of Conn & Louis (1956).

4. Summary

The delicate equilibrium between the body-fluid compartments poses many unanswered problems for the physiologist. It is clear, however, that the amounts of Na and K in the body, on which extracellular and intracellular fluid volumes largely depend, are closely regulated by the kidney. At present aldosterone is attracting much attention, for it has a greater effect on the urinary excretion of Na and K than any other known physiological variable. The exact stimuli for aldosterone secretion are still uncertain; their discovery will greatly help to solve the challenging problem of oedema.

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POTASSIUM DEFICIENCY AND THE KIDNEY

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1 Functional changes in the kidney
  a Glomerular function
  b Proximal tubular function
  c Distal tubular function
  d Functional changes produced by intracellular acidosis
2 Structural changes in the kidney
References

The effect of potassium (K) depletion on the kidney has been studied in the intact animal and by the tissue-slice technique. Although knowledge of this subject is still fragmentary, much interesting information has already been obtained. Potassium depletion can be produced experimentally by a K-deficient diet in combination with cation-exchange resins in either the sodium or the ammonium cycle. This technique has to be used with caution in man as severe K deficiency may possibly cause irreversible renal or cardiac damage. Much useful information has been obtained by studies of renal function and histology in diseases associated with K deficiency, e.g. prolonged diarrhoea, " potassium-losing nephritis ", and the recently described condition of primary aldosteronism (Conn, 1955). The correlation of functional and structural renal defects with the degree of depletion is of great importance. The latter can best be estimated by balance studies in man, and by muscle analysis in the experimental animal. Plasma K levels, electrocardiography, and isotope dilution have proved less satisfactory in the assessment of the severity of the K depletion.

1. Functional Changes in the Kidney

Renal functional defects in K deficiency may conveniently be classified under abnormalities of glomerular, proximal tubular, and distal tubular functions. In addition, certain changes seem to be due to intracellular acidosis rather than directly to K deficiency.

a. Glomerular Function

The glomerular filtration rate (GFR) is reduced in K deficiency, resulting in some retention of urea and non-protein nitrogen. Schwartz & Relman (1953) record a GFR of less than half the normal value in a patient deficient of about 500 m-equiv. K, and Muntwyler & Griffin (1953) report that the creatinine clearance of K-deficient rats is reduced to 60% of normal. The azotaemia in the rats may have been partly due to an acute renal circulatory failure, as there is often a profound fall in blood pressure (Freed & Friedman, 1950), but this can be excluded in man.

There is usually a slight proteinuria in severe K deficiency. It is not certain whether this is of glomerular or tubular origin, and it may be related to necrosis of tubular cells. Proteinuria occurs in most cases of primary aldosteronism, but in our cases was related more to hypertension and to excess circulating mineralo-corticoid rather than to the secondary K deficiency.

b. Proximal Tubular Function

Transport of *p*-aminohippurate (PAH) is reduced or completely inhibited in K-depleted kidney slices (Taggart, Silverman & Trayner, 1953). Renal function as determined by PAH excretion is therefore subnormal in K deficiency. Extraction of PAH (E_{PAH}) is reduced to about 70% (Schwartz & Relman, 1953), and the PAH clearance (C_{PAH}) is no longer a satisfactory index of renal plasma flow. The corrected renal blood flow is reduced less than the GFR. Tubular maximal secretory capacity for PAH (Tm_{PAH}) is also considerably lowered. The defects of proximal tubular function found in the Fanconi syndrome are unusual in K deficiency. Amino-aciduria has been recorded on one occasion (Denton, Wynn, McDonald & Simon, 1951), and Mahler & Stanbury (1956) report an abnormally high phosphate clearance which was restored to normal after K repletion.

c. Distal Tubular Function

Abnormalities of distal tubular function are especially characteristic of K deficiency, occurring after only mild grades of depletion. Repeated injections of deoxycortone produce a polyuria (Ragan, Ferrebee, Phyfe, Atchley & Loeb, 1940; Mulinos, Spingarn & Lojkin, 1941), which is now known to be due to increased urinary K loss. Polydipsia and polyuria occur after only a few days of experimental K depletion in dogs (Smith & Lasater, 1950) and rats (Spargo, 1954). In dogs, a peak diuresis was reported from the third to the seventh week of depletion. In rats, we have found that the diuresis persists until death occurs from hypokalaemic myocardial failure. Brokaw (1953) claimed that the kidneys remained sensitive to the action of the antidiuretic hormone, but we have found that pitressin injections do not affect the polyuria of rats severely depleted of K. The ability of the kidney to concentrate the urine is reduced by minor grades of K deficiency, a maximum specific gravity of 1010 having been found in patients deficient of 500 m-equiv. K (Schwartz & Relman, 1953). Renal concentrating power rapidly returns to normal after correction of the deficit. A similar defect is found in the nephropathy of primary aldosteronism, even in cases in which the kidney is histologically normal. An excess water load is not eliminated as rapidly by the K-depleted rat as by the normal (Brokaw, 1953). Thus the renal defect in K deficiency is similar to that of chronic nephritis, there being diminished power to respond efficiently either to water deprivation or to water excess. The fundamental difference is that the abnormality in K deficiency is easily reversed by K repletion.

In K deficiency the kidney is unable to produce a highly acid urine (Clarke, Evans, MacIntyre & Milne, 1955). This defect occurs with only minor deficits, but increases as the K

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loss becomes progressively more severe. There is no limitation of the amount of hydrogen ion (H^+) excreted, provided there is free availability of urinary buffer. The defect consists of an inability to produce a normal maximal concentration gradient of H^+ between urine and plasma. In man, the minimum urine pH is 4.5, corresponding to a concentration gradient of 800 to 1. After loss of only 300 m-equiv. K the minimum urine pH is 5.4 with a gradient of only 100 to 1. A similar defect in the secretion of gastric juice has been recorded in K-deficient rats (Carone & Cooke, 1953). Continuous ingestion of ammonium chloride produces maximal acidification of the urine on the second or third day, and thereafter there is progressive increase of urine pH which usually stabilizes at a value of about 5.3 by the seventh day (Wood, 1955). This secondary rise of pH is due to K depletion resulting from the acidosis, over 200 m-equiv. K being lost after ingestion of 8 g. ammonium chloride daily for one week.

The K-deficient kidney can efficiently retain K, very little being excreted in the urine (Black & Milne, 1952; Fourman, 1952), and thus the organism is protected from rapid and progressive depletion. In addition, stimuli which normally greatly increase urinary K loss, e.g. osmotic diuresis, hyperventilation, and ingestion of sodium bicarbonate, acetazolamide, or ammonium chloride, become relatively ineffective in K depletion, there being little rise of K excretion (Evans, Jones, Milne & Steiner, 1954; Clarke *et al.* 1955). Aldosterone, however, still increases urinary K loss, which accounts for the severe K deficiency found in primary aldosteronism (Conn, 1955; Aird, Milne & Muehrcke, 1956).

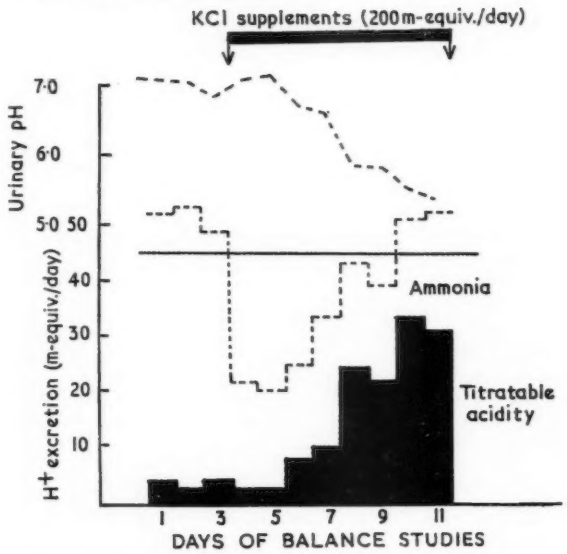
d. Functional Changes Produced by Intracellular Acidosis

Some renal functional abnormalities in K depletion appear to be caused by reduced intracellular pH of renal tubule cells rather than directly by the K loss. Potassium deficiency produces an intracellular acidosis with an extracellular alkalosis, there being an increased concentration gradient of H^+ across the cell membrane. Anderson & Mudge (1955) have shown that the intracellular bicarbonate of K-depleted tubule cells is reduced, thus conclusively proving an increase of cellular acidity. These reaction changes are primarily of metabolic origin, H^+ passing from the extracellular to the intracellular compartment in place of K (Cooke, Segar, Cheek, Coville & Darrow, 1952; Black & Milne, 1952; Orloff, Kennedy & Berliner, 1953). The extracellular alkalosis with increase of plasma bicarbonate must, however, be maintained by alteration of renal function. Berliner, Kennedy & Orloff (1951) postulate a competition between K and H^+ for exchange with sodium within the tubular lumen. Thus, if K exchange is reduced by depletion there would automatically be an increased transfer of H^+. An alternative explanation is that increased exchange of H^+ is directly due to increased concentration of this ion within the tubule cells. Roberts, Randall, Sanders & Hood (1955) have shown that more H^+ is in fact secreted by the renal tubules in K depletion, and thus more bicarbonate is reabsorbed. The resultant urine is acid in reaction despite increase of plasma bicarbonate, a phenomenon which has been termed the " paradoxical aciduria " of K depletion. Teleologically, this mechanism is certainly not paradoxical, since acid products of metabolism must be excreted in K deficiency as in the normal state.

The actual mechanism of H^+ excretion is, however, somewhat modified by K depletion. A greater proportion than normal is excreted as ammonia and less as titratable acid. Increased ammonia synthesis and excretion is due to a greater cellular content of the enzymes glutaminase and amino-acid oxidase (Iacobellis, Muntwyler & Griffin, 1954), which produce ammonia from glutamine and various amino acids. Since a similar increase occurs in ammonium chloride acidosis (Davies & Yudkin, 1952), and in acidosis produced by acetazolamide (Rector, Seldin, Roberts & Copenhaver, 1954), reduction of intracellular pH is probably the single effective stimulus. Normally, from half to two-thirds of excreted H^+ is eliminated as ammonia. In contrast, over 80% was excreted as ammonia in patients with intestinal K loss (Schwartz & Relman, 1953). We have found a similar high proportion in cases of K depletion due to primary aldosteronism (fig. 1). It has been claimed that a neutral or slightly acid urine is typical of this condition. In actual fact such a urine reaction is typical of any case of K depletion in which ammonia output accounts for the greater part of the normal excretion of H^+. If the urine were to become more acid, too much H^+ would be excreted and an alkalosis would inevitably result. Potassium repletion rapidly reduces ammonia output (fig. 1), and now the urine must become more acid if acid-base balance is to be maintained.

Excretion of organic acid, particularly citric and α-ketoglutaric acid, is dependent on systemic acid-base balance and not on urine pH (Evans, MacIntyre, Macpherson & Milne, 1957). Output of citrate falls with reduction of intracellular pH, e.g. in ammonium chloride and acetazolamide acidosis (Clarke *et al.* 1955) or in K depletion (Fourman &

FIG. 1. HYDROGEN-ION EXCRETION IN A CASE OF PRIMARY ALDOSTERONISM DEFICIENT OF 400 m-equiv. POTASSIUM, BEFORE AND DURING POTASSIUM REPLETION



Before repletion over 90% of excreted hydrogen ion is eliminated as ammonia; after repletion only one-third is excreted as ammonia. Potassium repletion causes a rapid fall in urinary pH.
The horizontal line represents the calculated acid-ash value of the constant daily diet, i.e. 45 m-equiv. hydrogen ion per day.

Robinson, 1953). This is not, however, a sensitive index of K deficiency in man. Citrate excretion is normal in mild degrees of experimental K depletion (Evans *et al.* 1954), and was within the normal range in a patient deficient of 400 m-equiv. K (Evans *et al.* 1957). Citrate excretion in the rat is more labile and is reduced after ten days of experimental depletion to about one-third the normal value. Rapid repletion with either K or rubidium bicarbonate produces a great increase of both citrate and α-ketoglutarate excretion to values far above the normal value (Cooke, Segar, Reed, Etzwiler, Vita, Brusilow & Darrow, 1954; Cooke, 1954; Evans *et al.* 1957). These variations of organic-acid excretion are not accompanied by any change in plasma organic acid, proving that the effect is of renal and not of metabolic origin. The fact that rubidium is as effective as K suggests that change in intracellular reaction is again the important factor.

In summary, changes of distal tubular function and those apparently due to increased cellular acidity are most characteristic of K deficiency. In particular, reduction of osmotic concentration power and failure to maintain a maximal concentration gradient of H^+ occur with only slight degrees of deficiency. The production of these chemical gradients requires energy which must involve the universal medium of biological energy exchange—the adenylic acid system. The important reversible reaction: phospho-enol-pyruvate + adenosinediphosphate $\rightleftharpoons$ pyruvate + adenosinetriphosphate, catalysed by the enzyme pyruvic phosphoferase, requires the presence of K in relatively high concentration (Kachmar & Boyer, 1953). Rubidium and caesium can replace K in this reaction, the order of efficiency being K > Rb > Cs. In K deficiency, oxidative phosphorylation reactions are depressed, and therefore less energy is available for the production of concentration gradients across the cell membrane.

2. Structural Changes in the Kidney

These changes in renal function produced by K deficiency are accompanied by considerable structural abnormalities. In the rat there is a gradual increase of kidney mass to about twice the normal value. This is not accompanied by any change in the percentage of water, fat, or protein, but there is tubular dilatation and increased mitosis of tubular cells (Spargo, 1954). Many pathologists (Follis, Orent-Keiles & McCollum, 1942; Kulka, Pearson & Robbins, 1950; Perkins, Petersen & Riley, 1950; Keye, 1952) have stressed the appearances of " hydropic degeneration " of tubule cells as being characteristic of K deficiency. These lesions are more prominent in the proximal than the distal tubular epithelium. They consist of large and usually single vacuoles containing neither fat nor glycogen. The nuclei of the cells are displaced towards the basement membrane, and the cytoplasm is ballooned inwards to project into the tubular lumen. In more severely affected areas there may be pyknosis of nuclei and actual cellular necrosis. Kulka *et al.* (1950) have described additional changes in the distal and collecting tubules of patients who have died in severe K deficiency. These consist of a granular degeneration and atrophy of tubular cells, and appear to be similar to the characteristic lesions in the renal papillae of K-deficient rats.

Spargo (1954), studying K deficiency in the rat, considered that granular degeneration of the cells of the collecting tubules was more prominent than the proximal hydropic changes. Our observations confirm this, and the results of parallel histochemical studies (Macpherson & Pearse, 1957) also suggest that the distal tubular changes are more important. After about 18 days of K depletion the cells of the collecting tubules in the distal three-fourths of the papilla become enlarged by the accumulation of numerous deeply eosinophilic, hyaline, spherical granules from 0.5 to 2.0 μ. in diameter (see Plate I, fig. B, compared with normal appearances, fig. A). This change is absent in the proximal fourth of the papilla, but every tubule in the distal three-fourths is involved, the lesion increasing in intensity towards the papillary tip. The granules are usually evenly scattered throughout the cytoplasm, but, in the most severely affected cells, large eosinophilic globules may occur. The epithelium covering the abnormal part of the papilla may be similarly affected, and there is some basophilia of the stroma between the affected tubules. These changes are invariably present to a gross degree in animals depleted for as long as three weeks, but are rapidly reversed by K repletion. The granules completely disappear one week after rapid K administration, but the cells remain somewhat enlarged.

Both the structural and functional lesions of the kidney in K deficiency are usually rapidly reversed by K repletion. It is possible, however, that severe and prolonged K deficiency may cause permanent irreversible renal damage. Fourman, McCance & Parker (1956) report renal failure with gross tubular dilatation many months after adequate K repletion in previously K-deficient rats. We have been unable to confirm this experiment, and thus it is probably not a constant phenomenon. In man, chronic pyelonephritis appears to be unduly frequent in cases of prolonged K deficiency. We have found histological evidence of pyelonephritis in patients with prolonged diarrhoea (Plate I, fig. C), and in severe primary aldosteronism (Plate I, fig. D). It is possible that the K-deficient kidney is more susceptible to bacterial infection than the normal. This problem is being investigated by studies of the effects of intravenous injections of pathogenic bacteria into normal and K-depleted rats.

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See Plate I facing this page for figs. A–D.—Ed.

* * *

PLATE I (FIGS. A—D)

POTASSIUM DEFICIENCY AND THE KIDNEY

(FIGS. A—D)

M. D. Milne, R. C. Muehrcke & B. E. Heard

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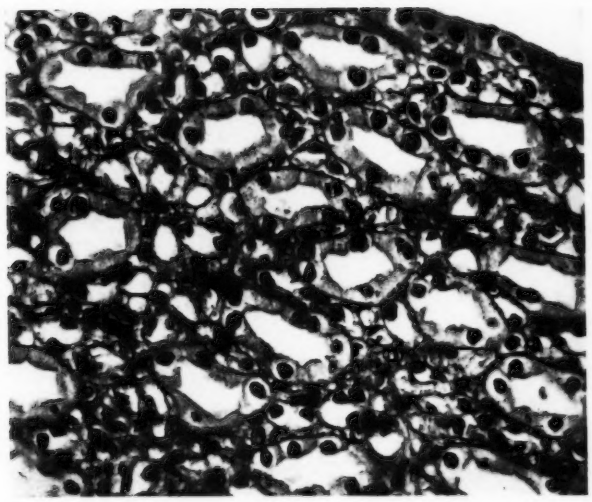

FIG. A. Collecting tubules from papilla of normal rat kidney

(Haemalum and eosin; magnification × 350)

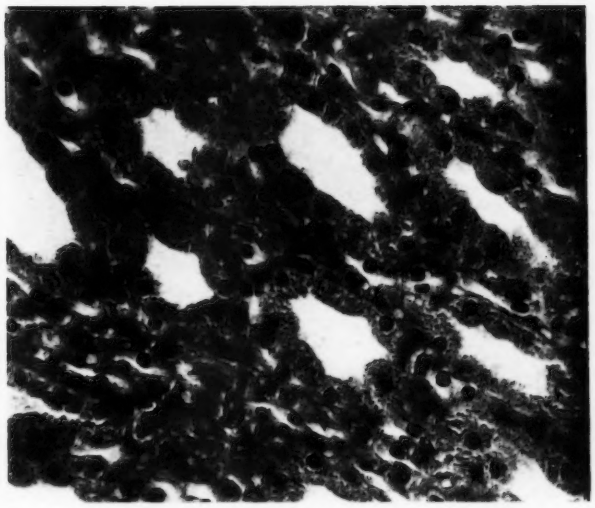

FIG. B. Collecting tubules from papilla of a potassium-deficient rat. Note the enlargement and granularity of the tubule cells

(Haemalum and eosin; magnification × 350)

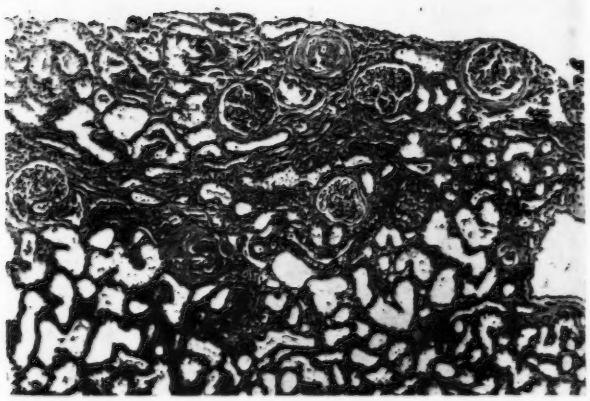

FIG. C. Section of kidney obtained by aspiration renal biopsy from a case of prolonged diarrhoea due to over-use of laxatives. Characteristic changes of chronic pyelo-nephritis are seen

(Haemalum and eosin; magnification × 58)

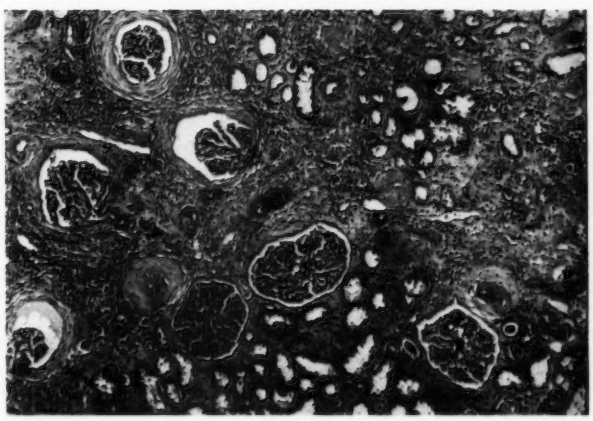

FIG. D. Section of kidney obtained during operation of unilateral adrenalectomy in a case of primary aldosteronism with severe and prolonged potassium deficiency. There is very severe chronic pyelonephritis

(Haemalum and eosin; magnification × 58)

PLATE II (FIGS. A—H)

HISTOCHEMICAL CHANGES IN THE POTASSIUM-DEPLETED KIDNEY

(FIGS. A—H)

C. R. Macpherson & A. G. E. Pearse

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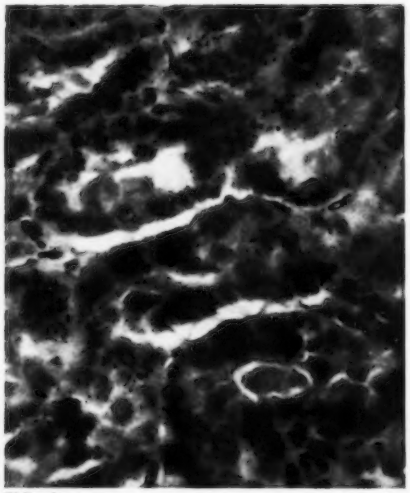

FIG. A

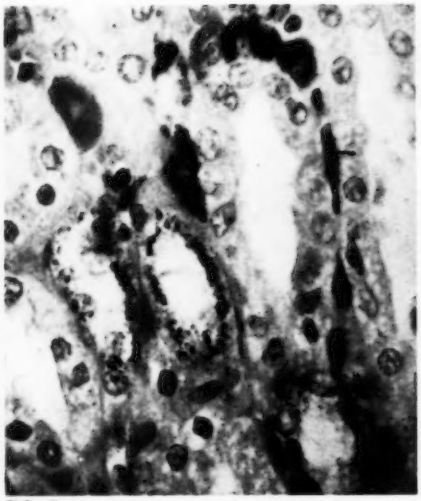

FIG. B

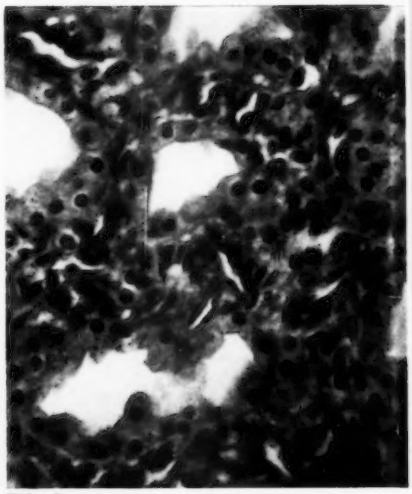

FIG. C

FIG. D

FIG. E

FIG. F

FIG. G

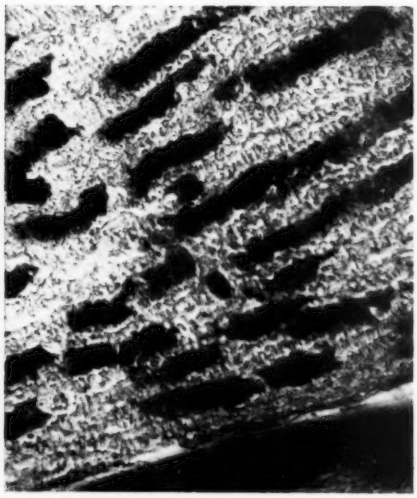

FIG. H

HISTOCHEMICAL CHANGES IN THE POTASSIUM-DEPLETED KIDNEY

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A. G. E. PEARSE M.A. M.D. D.C.P.

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The only recorded histochemical studies on the kidneys of potassium-depleted rats are those of Spargo (1954) who reported a decrease of alkaline phosphatase. Parallel studies with homogenized tissue indicated a decrease to about 50% of the control values. No histochemical change was observed in esterase or acid phosphatase. Using tissue homogenates, Iacobellis, Muntwyler & Griffin (1954) found a significant increase in glutaminase and carbonic anhydrase. If the animals were also protein-deficient, however, these enzymes were decreased. Arginase and D-amino-acid oxidase activity was apparently increased, but, when calculated per unit of kidney nitrogen, activity was within normal limits.

In the hope of demonstrating and localizing changes in renal function in potassium (K) depletion, we applied histochemical techniques for the following enzymes or enzyme systems:

i. Alkaline phosphatase (Pearse, 1953).
ii. Acid phosphatase (Grogg & Pearse, 1952).
iii. Non-specific esterases (Holt & Withers, 1952).
iv. Succinic dehydrogenase (Rutenberg, Wolman & Seligman, 1953).
v. Triphosphopyridine nucleotide (TPN) and diphosphopyridine nucleotide (DPN) diaphorases (Farber, Sternberg & Dunlap, 1954).
vi. Phosphorylase (Takeuchi & Kuriaki, 1955).
vii. 5-Nucleotidase (Pearse & Reis, 1952).

In each case the references given include the techniques used, with appropriate modifications. Potassium-depleted rats and suitable control animals were studied 8, 18, 20, 21, 22, 24 and 27 days after depletion had begun, and potassium-repleted animals were also studied.

Results

i. *Alkaline Phosphatase.* No significant change was observed. On the 22nd, 24th and 27th days some (abnormal) activity was present in the dilated tubules of the inner medulla.

ii. *Acid Phosphatase.* A slight increase was noted on the 18th day, and much more on subsequent days, in the tubules of the inner medulla and even more in those of the papilla (collecting tubules).

iii. *Esterases.* Increased activity in the proximal convoluted tubules, and possibly in the descending loops of Henle, was noted as early as the 8th day. On subsequent days this became progressively greater; by the 24th day the enzyme was present in large globules and masses (Plate II, fig. A) contrasting sharply with the normal fine granules found in rats of this age and sex. A diffuse intracellular localization also became apparent; this is never seen in normal animals. At the same time the enzyme appeared in large amounts in the inner medulla and in the papilla. In these sites the greatest increase was due to the appearance of cells, normally substantially esterase-free, which were tentatively identified as thin loops (Plate II, fig. B). At the tip of the papilla there was an increase in the normal enzyme of the lining epithelium, and in the swollen granular convoluted tubule a granular esterase appeared. This was associated with the appearance of esterase in cells similar to those observed in the medulla (resembling thin loops), but some at least of these cells may belong to the interstitial tissue (Plate II, fig. C). No restoration towards the normal appearance was seen after brief therapy to correct K depletion.

iv. *Succinic Dehydrogenase.* The normal amount and distribution of succinic dehydrogenase, with its abrupt disappearance at the junction of medulla and papilla (Plate II, fig. D), was invariably present.

* Eli Lilly Research Fellow.

PLATE II. HISTOCHEMICAL CHANGES IN THE POTASSIUM-DEPLETED KIDNEY

FIG. A. Cold formalin-fixed 5-μ. paraffin section (cortex). Shows large esterase-containing bodies in proximal convoluted tubules. 27 days' depletion
(Stain: 5-Bromoindoxyl acetate, Carmalum; magnification × 430)

FIG. B. Cold formalin-fixed 5-μ. paraffin section (medulla). There is some increase of esterase in the fine granules in the tubules and a great increase in cells lying between them. 27 days' depletion
(Stain: 5-Bromoindoxyl acetate, Carmalum; magnification × 335)

FIG. C. Cold formalin-fixed 5-μ. paraffin section (papilla). Clefts lined by cells containing a strong esterase lie between the collecting tubules. 27 days' depletion
(Stain: 5-Bromoindoxyl acetate, Carmalum; magnification × 370)

FIG. D. Fresh frozen 20-μ. section (cortico-medullary junction). Shows reaction for succinic dehydrogenase. 27 days' depletion
(Stain: Ditetrazolium chloride; magnification × 130)

FIG. E. Fresh frozen 20-μ. section. Reaction for DPN diaphorase in the medulla of a control rat. Incubation 2 hours
(Stain: Ditetrazolium chloride; magnification × 130)

FIG. F. Fresh frozen 20-μ. section. For comparison with fig. E. DPN diaphorase in rat depleted for 22 days. Incubation 2 hours
(Stain: Ditetrazolium chloride; magnification × 130)

FIG. G. Fresh frozen 20-μ. section. Reaction for TPN diaphorase in the medulla of a rat depleted for 22 days
(Stain: Ditetrazolium chloride; magnification × 130)

FIG. H. Fresh frozen 20-μ. section. A strong phosphorylase in the collecting tubules and in the papillary and pelvic epithelium of a rat depleted for 24 days
(Phosphorylase reaction; magnification × 160)

v. *DPN Diaphorase.* From the 18th day onwards a progressively greater diminution of this enzyme was observed in the medulla and papilla, where normally the thick loops are the site of very active reduction of tetrazolium (Plate II, figs. E, F). A very striking increase was noted after K depletion had been corrected.

TPN Diaphorase. In the K-deficient animals, a particularly striking increase in this enzyme was noted in the hypertrophied medullary pyramid (Plate II, fig. G). This showed itself as a visible reduction of the colourless tetrazolium salt to a blue formazan pigment before the sections could be transferred to the usual anaerobic environment for incubation at 37° C. After brief K-repletion therapy the normal rate of reaction was restored. We believe incidentally that some of the strongly stained, transversely orientated, fusiform and stellate cells of the papilla, regarded by Farber *et al.* (1954) as possible contractile elements, are the normal cells of the thin loops which are compressed from cuboidal to fusiform shape by the tubular proliferation which occurs in K deficiency.

vi. *Phosphorylase.* This enzyme occurs in the pelvic and papillary epithelium, in the collecting tubules and distal convoluted tubules, and in the lumen of another set of tubules in the medulla. These are likely to be ascending loops. Differences between the K-deficient and control groups were small; in the papilla, however, there appeared to be a stronger enzyme in the deficient group (Plate II, fig. H).

vii. 5-*Nucleotidase.* No significant difference was found between control and K-depleted animals.

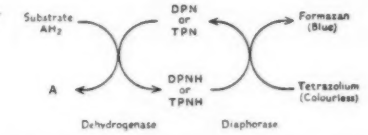
Discussion

Our results suggest that, although the functional changes of K deficiency affect all parts of the kidney, they are largely confined to the medulla and particularly to the inner medulla and papilla. Involvement of the cortex is suggested only by the changes in esterase.

The significance of the various changes is not easily determined. It seems likely, however, that the raised acid phosphatase and phosphorylase simply indicates a rise in phosphorylating capacity, since Davison-Reynolds, Barrueto & Lemon (1955) have shown that prostatic acid phosphatase is responsible for transphosphorylase activity, transferring phosphate to suitable alcohols.

We believe that the difference between our results and those of Spargo (1954) in respect of alkaline phosphatase can be accounted for by the fact that his animals were protein-deficient as well as K-depleted, and we do not consider changes in this enzyme in any way significant.

The two diaphorases act, in the artificial system provided by the incubation medium, as carriers of hydrogen from reduced coenzyme I (DPNH) to the tetrazolium salt, the source of hydrogen being the substrate in the first instance. Since all the necessary factors except the diaphorase are present in the medium, the constantly diminished reaction



The substrate AH_2 which acts as the original hydrogen donor for the reduction of DPN or TPN can be succinate, malate, alcohol, glucose or any other compound for which an endogenous dehydrogenase is present or has been added to the medium.

for DPN diaphorase means that a smaller amount of this enzyme is present or that its activity is blocked. The great increase in TPN diaphorase activity must be due to an actual increase in the amount of this enzyme. TPN can be formed from DPN in the presence of phosphate (or adenosinetriphosphate) and an enzyme which is present in yeast. It is possible, therefore, that in the medulla of the K-deficient kidney conditions are such that much or all of the DPN present in the incubating medium is converted to TPN and thus inactivated as far as its primary function is concerned. In the cortex, in the absence of increased phosphorylating capacity, normal DPN diaphorase activity would be present. Alternatively, the observed effects may simply be due to a fall in the level of the flavoprotein DPN diaphorase in the K-deficient medulla, with a coincident rise in the flavoprotein TPN diaphorase.

If the true in-vivo functions of the non-specific indoxyl esterase were known, interpretation of the esterase picture would be much easier. The possibility that the esterase is really an intracellular peptidase must be borne in mind. Making assumptions based largely on our observations of esterase changes and their functional correlation in other organs, we consider that in K depletion there is increased intracellular metabolism of lipids or protein, particularly in the cells of the thin loops, but perhaps also in the interstitial cells. It is apparent that the intracellular changes which manifest themselves as changes in esterase distribution represent a more slowly reversible abnormality than those affecting the diaphorase systems. The reason for this it is at present impossible to guess.

ACKNOWLEDGEMENTS

We have to thank Dr H. Harms and Farbenfabriken Bayer, Leverkusen, for the kind gift of a pure sample of ditetrazolium chloride.

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MICRODISSECTION OF THE NEPHRON IN DISEASE

E. M. DARMADY M.A. M.D. F.R.C.P.

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1 Introduction
2 Acute tubular necrosis
  a Changes in relation to phase of the disease
  b Random distribution of lesions
  c Interstitial oedema
  d Anuria in homotransplanted kidneys
3 Lignac-Fanconi disease (cystinosis)
4 Congenital familial nephrosis
5 Infantile pyelonephritis or interstitial nephritis
6 Nephrocalcinosis
7 The kidney in potassium-losing states
References

In the past, histologists and morbid anatomists have been faced with many difficulties when studying the kidney in disease. Unlike other organs the kidney is composed of a number of units, each complete in itself, yet liable to develop localized and focal changes, which may vary from nephron to nephron. For, in disease, the tubules are liable to become distorted, accurate localization may be impossible and, at the same time, micro-anatomical lesions may escape detection. Microdissection of the kidney overcomes many of these difficulties, for it allows the nephron to be studied in three dimensions, the unit to be traced from start to finish, and the exact position of pathological lesions to be localized.

1. Introduction

It was for these reasons that we decided to make a study of the nephron by microdissection, in the hope that this might clarify some of the problems in renal pathology; we were being careful at the same time to correlate our findings with the biochemical changes found in life.

Technique of Maceration

In our experience, tissue fixed in neutral formol saline for at least a week has proved to be the best for this technique. Difficulty has been found with tissues fixed in alcohol, as the nephron becomes brittle, and is liable to break. Wedge-shaped pieces of tissue are cut from the coronally bisected kidney to include the cortex and medulla in continuity from the capsule to the tip of the pyramid. (Unfortunately many of the kidneys sent to us have been cut by other methods.) The pieces of tissue vary in size according to the size of the kidney, but are never more than 2 mm. in thickness. Lately it has proved possible to obtain a number of complete nephrons in 60-μ. thick slices; thus the method is applicable to biopsy material. A number of representative samples from various areas of the kidney are transferred to a wide-mouthed glass jar with a well-fitting stopper, containing concentrated hydrochloric acid (sp. gr., 1.150–1.160 at 20° C.) at room temperature (about 20°–22° C.) for an average time of 48 hours. A portion of slide, suitably marked with a diamond, is added to serve as an identifying label. The temperature is found to cause variation in the length of time required for adequate maceration, the process taking less time in warmer weather. The maceration can be greatly accelerated by placing the container in an incubator (37° C.), but in our hands this has proved unsatisfactory, because the speed of the maceration may cause early disintegration of the material. Experience is usually necessary to decide whether the kidney has become sufficiently soft but, as soon as the material is ready, it is washed with three or four changes of distilled water. We have found it easier to dissect macerated tissue which has been left in distilled water for about 24 hours than tissue recently rinsed. The tubules are teased apart in distilled water in a Perspex dish, $8 \times 5 \times 0.5$ cm., either by Singer microdissectors or by needles mounted in wooden handles, under a binocular stereoscopic microscope at a magnification of 20 diameters, with incident illumination from powerful lamp and Lucite rod. The most satisfactory needle for the purpose has proved to be the ordinary domestic sewing needle (gauge 10 or 12).

For photography or record, an isolated nephron or tubule is removed by mounting it on a glass cover-slip previously placed to one side of the dish. The tubule is so arranged that it is adherent throughout its length to the glass. The cover-slip is then lifted out of the water, and inverted into a drop of water in the centre of a slide previously prepared with a smear of vaseline along each edge. The fluid mount is then sealed round the edges with Glyceel (George T. Gurr Ltd., London), and allowed to dry before being photographed. We have not used a staining method to differentiate the material, as we have found that adequate contrast can be obtained by phase microscopy and photography. If staining is required, the stain is placed to one side of the cover-slip and allowed to diffuse through the fluid medium. The process can be accelerated by drawing the stain through by small portions of filter paper. In our hands the stain is inclined to be taken up irregularly and may produce artifacts. Many macerating agents other than hydrochloric acid have been tried by us, including concentrated nitric acid, sulphuric acid, acetic acid, sodium hydroxide, and various enzymes—among these papain, pepsin, trypsin, hyaluronidase, and *Clostridium welchii* toxin, but without success.

The technique described has been derived from Oliver, MacDowell & Tracy (1951) who found that temperature was the most important variable in the technique, although the size of block, degree of fibrosis and length of time of fixation also played a part. Variations in the strength of the hydrochloric acid have been suggested (Henle, 1862; Peter, 1909; Marshall & Grafflin, 1928). Some have found it necessary to perfuse the kidney first (Huber, 1911; Traut, 1923; Stewart, 1927).

We have used microdissection to investigate a number of conditions in which either the localization of pathological lesions has been equivocal, for example acute tubular necrosis, salt-losing nephritis, aldosteronism; or where micro-anatomical lesions have been discovered in the course of investigating obscure nephropathies, which we propose to present in further detail.

2. Acute Tubular Necrosis

The recognition by Bywaters (1941) of a new syndrome following crushing injuries led to considerable interest in all forms of acute uraemia. The histological appearances in fatal cases were fully described by Dunn, Gillespie & Niven (1941), and by Bywaters & Dible (1942). They drew attention to the changes seen in the boundary zone, the loops of Henle and collecting tubules, and the presence of tubular ruptures often associated with veins. Later Lucke (1946), on reviewing the syndrome, was impressed by the changes seen in the distal and collecting tubules, and suggested that the syndrome should be known as "lower nephron nephrosis". Foy, Altmann, Barnes & Kondi (1943), Maegraith, Harvard & Parsons (1945), Lucke (1946), and Darmady (1948) found that anuria

could follow a number of aetiological factors, such as shock, electrolyte-water imbalance, and the presence of intravascular haemolysis, and yet that at death similar renal changes could be found, and were often indistinguishable one from another.

In the meantime, Muirhead, Haley, Haberman & Hill (1948), and Bull, Joekes & Lowe (1950) noticed that the condition appeared to have four phases: i. The onset or precipitating phase; ii. anuric phase; iii. diuretic phase; iv. late diuretic phase. Biochemical tests suggested that, in addition to a reduction in renal blood flow, there is considerable tubular dysfunction, which was somewhat at variance with opinions expressed previously. However, Oliver *et al.* (1951) showed by microdissection that, in acute anuria produced by shock, there was considerable focal damage to the whole nephron including the proximal tubule, and that this was accompanied by tubular ruptures, which they called "tubulorhextic lesions". They also found that, where nephrotoxic poisons such as mercury, glycol, etc., were the aetiological factors, there was a specific localized epithelial necrosis of the tubule according to the nephrotoxin used. These they termed ischaemuric lesions. From a clinical standpoint, however, whatever the aetiological factor, the course of the illness was identical.

Dible (1953), in reviewing a personal series of 62 cases of anuria, divided them into two main groups on histological grounds: those in which there was localized tubular necrosis due to nephrotoxins of a chemical nature, and those in which there was selective damage to the nephron most marked in the boundary zone of the distal and collecting tubules. It is this latter group which apparently have their origin in severe ischaemia or which are at times accompanied by intravascular haemolysis. Dible further carried out an extensive study of cases of focal damage to the nephron and traced the occurrence of the lesions in order of their appearance from the date of onset. In amplification of the studies made by Oliver *et al.* (1951) and Dible (1953), a study has been made both by microdissections and from histological preparations of 53 kidneys from patients dying of anuria with similar aetiological factors, but not including known nephrotoxins.

a. Changes in Relation to Phase of the Disease

In general, our findings confirm those of Oliver *et al.* (1951).

Onset Phase

In the first or onset stage lasting up to 36 hours, little or no change can be detected in histological preparations, except in the glomerulus which, although anatomically unaffected at all stages of the syndrome, is often engorged with blood. However, by microdissection many nephrons show focal points of disarrangement of tubular epithelial pattern and loss of translucency. These changes are mostly confined to the proximal tubules, but do not affect all nephrons equally. The fact that nephrons are not equally affected is thought to be of importance and will be referred to later.

Anuric or Oliguric Phase

Tubular change. During the early stages of anuria or oliguria histological preparations show that the glomeruli are seldom filled with blood. The most constant change after 36 hours is the appearance of transparent casts in the lower end of the proximal tubule and loops of Henle. Microdissection again shows that there is tubular epithelial disarrangement and focal necrosis, whilst the lumen of the proximal tubule becomes visible (see Plate III, fig. A and compare with fig. B, which shows a normal proximal tubule). Again not all nephrons are affected equally and indeed some may be considered completely normal, although at this stage only small quantities of urine may be excreted. Early evidence of regeneration of the epithelium is shown by heaping of cells (see Plate III, fig. C), which often occurs in the same nephron; the latter may also show evidence of necrosis in some other area (Plate III, fig. C). In places, localized dilatation of the tubule, frequently in the lower end of the proximal tubule, can be seen without evidence of cast formation lower in the nephron (Plate III, fig. D). How this occurs is not certain, but it may be because the wall of the tubule becomes softened and dilated. The predominant changes are, however, seen lower in the nephron, with cast formation in the loops of Henle, the distal and collecting tubules. Here the lumen is clearly visible and dilated, for the epithelium has become shrunken and flattened.

Tubular ruptures. During the mid-point of the anuric phase, ruptures of the tubular wall appear which are thought to correspond to the tubulovenous or tubular interstitial ruptures

PLATE III. MICRODISSECTION OF THE NEPHRON IN DISEASE

FIG. A. Proximal tubule from a patient with post-operative shock of 36 hours' duration. Note focal disarrangement of epithelium and dilatation of the lumen (magnification × 400)

FIG. B. Normal proximal tubule. Note that the epithelial arrangement is regular and the lumen is not visible (magnification × 300)

FIG. C. Proximal tubule from a patient with post-operative shock of 5 days' duration, and possible sulphonamide reaction in the oliguric phase. Note the regeneration with heaping of epithelial cells (magnification × 240)

FIG. D. Lower end of proximal tubule from the case illustrated in fig. A. Note the dilatation of the lumen without cast formation (magnification × 100)

FIG. E. Distal tubule with multiple cast formation and tubular rupture, from a patient with ante-partum haemorrhage, dying 9 days after onset, in the late anuric phase (magnification × 240)

FIG. F. Distal tubule from the case illustrated in fig. E, with multiple cast formation without distortion or dilatation of the tubule (magnification × 180)

FIG. G. Normal distal tubule with a small diverticulum (a feature of the distal tubule) (magnification × 200)

FIG. H. Glomerulus over-distended with Indian ink, showing the atrophy and narrowing of the first part of the proximal tubule from the homotransplanted kidney of dog in anuria (magnification × 160)

FIG. J. Normal human glomerulus, showing the wide neck and regular arrangement of the epithelium and nuclear spaces of the proximal tubule (magnification × 120)

MICRODISSECTION OF THE NEPHRON IN DISEASE

(FIGS. A—S)

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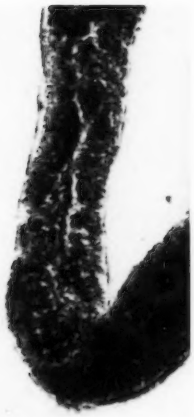

FIG. A

FIG. B

FIG. C

FIG. D

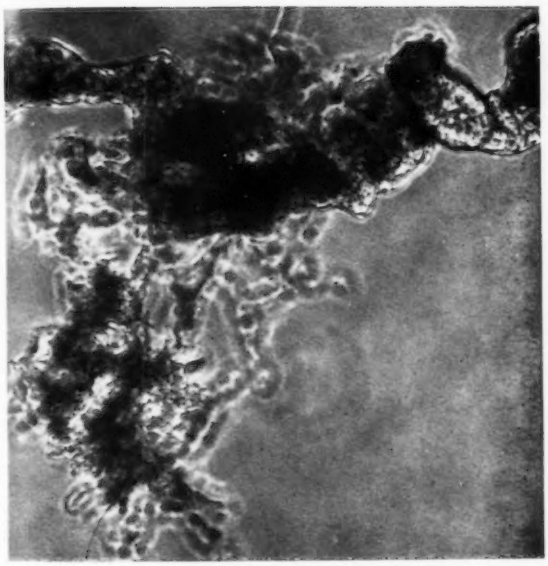

FIG. E

FIG. F

FIG. G

FIG. H

FIG. J

FIG. K

FIG. L

FIG. M

FIG. N

FIG. O

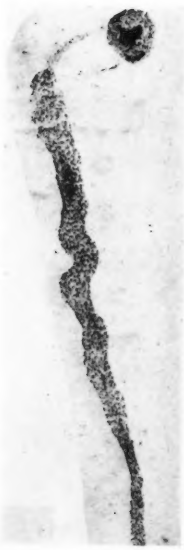

FIG. P

FIG. Q

FIG. R

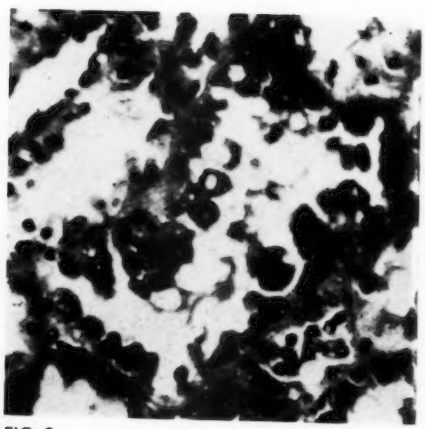

FIG. S

seen in histological preparations. The ruptures, into which protein-like material may leak, are found in all parts of the nephron: the loops of Henle, the proximal, distal and collecting tubules being equally involved (Plate III, fig. E).

Cast formation. It was noticed that casts were seen in various sites in the same nephron and that the cast material does not distend the tubule, provided that the wall of the tubule is intact. The cast takes up the shape of the lumen and is in close apposition to the outline of the epithelium (Plate III, fig. F, which is compared with the normal in Plate III, fig. G). As the disease progresses, so are casts found more frequently in the lower part of the nephron; the majority appear to be in the distal and collecting tubules, but some may also be found in the lower end of the proximal tubule. Dilatation of the tubule above the cast was not found. In places where the epithelial rupture has occurred, casts of protein-like material may be found in the interstitial tissue.

Diuretic Phase

In the diuretic phase, evidence of regeneration is seen at many parts of the proximal, distal and collecting tubules. The epithelial pattern becomes regular, although the distal and collecting tubules lag behind the regenerative progress made by the proximal tubule. It is noticeable in the distal and collecting tubule that an infiltration of the casts by polymorphonuclear leucocytes and macrophages is seen in histological preparations.

b. Random Distribution of Lesions

It is clear from microdissection studies that the distribution of the lesions is very variable, and it would seem that some of the tubules may show multiple casts from one end to the other. Others may be completely clear, and the tubular arrangement of the epithelium not unduly disarranged. On the other hand, some may show ruptures of the walls and atrophy in the final stages. It is clear that some of the nephrons are so damaged that they are unlikely to be capable of future function. The random distribution of the lesions and the apparent escape of some of the nephrons may be the reason why some of the nephrons are capable of secreting urine during the oliguric phase.

c. Interstitial Oedema

Dible (1953) has suggested that, in the anuric or in the recovery phase, there may be interstitial oedema with early fibrosis, most marked in the boundary zone, and that this may result in a " relative functional bar " to the passage of filtrate. From microdissection there is no evidence to suggest that a raised intra-renal pressure has caused a collapse of nephrons either in the proximal or the distal tubules, and in fact the majority of tubules show focal areas of regeneration. Some support for this also is given by de Wardener (1955) who measured the intra-renal pressure in dogs in which experimental acute tubular necrosis had been produced, and found that it was unaltered.

d. Anuria in Homotransplanted Kidneys

Another form of anuria which has recently interested us is that which follows homotransplantation of the kidney. Usually homotransplanted kidneys of dogs are capable of secreting urine up to 4–6 days after transplantation. At about this time there is abrupt cessation of urine flow, and unless the transplanted kidney is removed rapidly the animal dies. Examination of the nephron shows that the first part of the tubule—or glomerular neck—becomes narrow and devoid of epithelium (Darmady, Dempster & Stranack, 1955). This may occur before the cessation of urine flow, and is clearly not an obstruction to filtration (see Plate III, fig. H, and compare with J, which shows a normal glomerulus). This is confirmed by the fact that in cortisone-treated animals the neck can be partly preserved, and yet the animals invariably develop anuria. However, injection of Indian ink into the renal circulation at the time of anuria both in untreated and cortisone-treated homotransplanted kidney shows that the glomeruli are overfilled with blood, suggesting that the tone of the capillary tuft has been lost, the blood flow is sluggish or even stagnant, and glomerular filtration is minimal. Since the kidneys have been transplanted, the observed change cannot be mediated through the nervous system. We have also examined two cases of homotransplantation of the human kidney, and have found similar changes. Anuria occurred in both patients.

3. Lignac-Fanconi Disease (Cystinosis)

In this syndrome, the evidence of glycosuria with increased phosphaturia, aminoaciduria, and increased urine potassium (all occurring in the presence of normal or lowered plasma levels), together with an inability to acidify the urine, suggested that there might be a structural defect of the renal tubule; one

PLATE IV. MICRODISSECTION OF THE NEPHRON IN DISEASE

FIG. K. Glomerulus and narrow neck joining the proximal tubule from a patient with Lignac-Fanconi disease (a swan-neck lesion) (magnification × 124)

FIG. L. Glomerulus and "hypotrophic" proximal tubule from a patient with Lignac-Fanconi disease (magnification × 140)

FIG. M. The healed end of an aglomerular atrophic proximal tubule. Note the apparently viable epithelium of the tubule (magnification × 500)

FIG. N. Complete tubule from a foetus of 28 weeks' gestation. Note the absence of the loop of Henle and the lack of differentiation (magnification × 88)

FIG. O. An almost complete proximal tubule from a premature infant of 36 weeks' gestation. Note the absence of convolutions, and the under-development of the tubule (magnification × 100)

FIG. P. An almost complete proximal tubule from an infant with congenital familial pyelonephritis developing terminal nephrosis. Note the absence of convolutions and the dilated tubules (magnification × 50)

FIG. Q. A portion of proximal tubule with three plaques of opaque calcium-like material, both intra-tubular and adherent to the tubular surface (magnification × 240)

FIG. R. Proximal tubule obtained by biopsy from a patient with Conn's syndrome. Note the marked vacuolation of the tubular epithelium (magnification × 300)

FIG. S. The marked vacuolation of the tubular epithelium from a patient with adrenal carcinoma and potassium depletion (magnification × 400)

in the proximal part would be adequate to account for all these facts. Indeed this was subsequently confirmed by microdissection, for, of the 11 cases examined where the clinical history conformed strictly to that laid down for Lignac-Fanconi disease (cystinosis), as shown in Table I, eight showed

TABLE I. LIGNAC-FANCONI DISEASE (CYSTINOSIS)
(personal series studied by Darmady & Stranack)

Case number	Donor of specimen	Age of patient	Findings	Remarks
1	Dr Dent	14 months	Swan-neck lesion	
31	Dr Baar	9 years	Swan-neck lesion	Case number 9*
42	Dr Baar	15 months	Hypotrophic	Sibling of case numbers 15 and 16* and of our case number 134
60	Dr Fagg & Dr Dent	—	Swan-neck lesion	
81	Dr Baar	18 months	Hypotrophic	
103	Dr Craig & Dr Dent	5½ years	Swan-neck lesion	Alcohol-fixed, full dissection not possible
107	Dr Siebenmann	2½ years	Swan-neck lesion	
110	Dr Bodian	4½ years	Swan-neck lesion	
113	Dr Meek	5½ years	Swan-neck lesion	Remainder of proximal tubule distorted with fat deposition
134	Dr Baar	6½ years	Hypotrophic	Case number 16*; sibling of our case number 42. Alcohol-fixed, full dissection not possible
137	Dr Bradley Watson	8 years	Swan-neck lesion	Sibling of our case number 60

* Baar, J. S. & Bickel, H. (1952) *Acta paediat., Stockh.* Suppl. 90

that the proximal tubule was shorter than normal, and that the first part of the proximal tubule was replaced by a thin and narrow neck for about a third of its length (Plate IV, fig. K). Because of its thin and narrow neck, and its attachment to the glomerulus, it has been termed "swan-neck" lesion. In the remaining three, the proximal tubule was found to be hypotrophic throughout its length (see Plate IV, fig. L), i.e., it was narrower and shorter than normal. The degree of hypoplasia and tubular abnormality seemed to depend upon the genetic linkage of the particular family concerned. Some of these findings have been previously reported (Clay, Darmady & Hawkins, 1953; Darmady, 1954; Darmady & Stranack, 1954, 1955).

Oliver (1939) found that a small proportion of nephrons in Bright's disease showed a thin and narrow neck variable in length ("goose-necks"), a number of which were aglomerular. In contrast to the goose-neck lesions seen in Bright's disease is the fact that almost all the nephrons in the Lignac-Fanconi syndrome are of the same type, either swan-neck lesions or hypotrophic. Aglomerular nephrons were not seen in any of the cases examined. It is just possible that the presence of aglomerular tubules might have been obscured by the massive fibrosis, but we believe this is unlikely, since it was possible to find aglomerular nephrons without difficulty in other diseases, such as in Bright's and allied diseases (see Plate IV, fig. M).

In addition to the cases of Lignac-Fanconi disease previously described, we have also been able to examine the kidneys of three other patients who presented in life with glycosuria, phosphaturia, hypopotassaemia, abnormal aminoaciduria and dwarfism, but, with one exception, without cystinosis. The first patient (case number 2), aged 46, previously reported by Clay, Darmady & Hawkins (1953), and the second patient (case number 121), aged two years, were both found to have a swan-neck lesion of the proximal tubule. In the latter case, the upper two-thirds of the tubule was affected and amounted almost to a hypotrophic lesion; it was interesting to note that cirrhosis of the liver was also present. The third case (number 108), in which the history was atypical of Lignac-Fanconi disease, was also stated to have cystinosis but was unusual in that the symptoms of the disease started from birth. Examination of the nephron from this kidney showed a hypotrophic lesion.

4. Congenital Familial Nephrosis

We were also asked to investigate the kidneys of two siblings who died of a nephrotic-like syndrome in early infancy. Their parents were first cousins, as were their paternal grandparents. These cases are being reported by Pugh, Giles, Darmady, Stranack & Wolfe (unpublished). Both were in males, who died at the age of six and five months (case numbers 65a and 65b). Microdissection showed that the proximal tubule was replaced by a swan-neck lesion but, in addition, the lower two-thirds of the tubule was dilated, the lumen ballooned and the epithelium flattened. In another male infant (case number 111), who died at the age of 11 weeks of a nephrotic-like syndrome, microdissection showed a mixture of proximal tubules of the premature type (described below) and swan-neck lesions. Again the parents were related. However, there was no evidence during life of phosphaturia or aminoaciduria of a distribution like that found in Lignac-Fanconi disease.

Nephrons of the Neonatal Period

So far 12 kidneys of normal neonatal infants dying of causes other than renal disease have been examined. Their ages varied from 28 weeks' gestation to 8 months. Our findings confirm those described by Peter (1927). In a foetus of 28 weeks' gestation, we found that the loop of Henle is not visible, and that the proximal and distal tubules are continuous without any evidence of differentiation (Plate IV, fig. N). At 36 weeks' gestation the nephron is differentiated, and the proximal and distal tubules show few convolutions (Plate IV, fig. O). The loops of Henle are short, but are of constant diameter. The nephrons at full term show that the proximal tubule is well convoluted, and that the descending and ascending limbs of the loop of Henle are easily recognized. The nephron, while resembling that of the adult in pattern, is still considerably shorter. We did not encounter any proximal tubules of the swan-neck or hypotrophic type. It is probable, however, that the proximal tubules in early infancy are shorter in proportion to those of the adult; so far no accurate measurements have been made.

5. Infantile Pyelonephritis or Interstitial Nephritis

We have also examined the kidneys of four infants who all presented with pyelonephritis. Two of these were siblings aged four and a half and four months (case numbers 80a and 80b)[1]; the third was aged 24 days, and the fourth aged 21 weeks. In the first three cases, microdissection of the nephrons showed that there was considerable fibrosis, and that the proximal tubules closely resembled those of a premature infant (see Plate IV, fig. P), that is to say, they were lacking in convolutions and shorter than normal. In the fourth case, the nephrons did not apparently differ from those of a normal control of similar age.

6. Nephrocalcinosis

We have attempted to dissect a number of kidneys which have shown nephrocalcinosis, with a view to determining the site of calcium deposition. With the exception of one case, we have been hampered because the calcium " granuloma " has caused considerable disorganization of the tubules and has resulted in many of the tubules becoming matted together, with the result that accurate localization of the primary lesion became impossible. However, in one case (number 123), we were able to find microdeposits of calcium-like material scattered through the kidney. This case, reported by Nordin & Fraser (1953), had been regarded as one of vitamin D resistant rickets with gross osteomalacia, and had in the past received large doses of vitamin D, but the patient had not responded to treatment. The serum calcium and phosphate levels could not be maintained at normal levels, and the patient continued to show excessive losses of phosphate in the urine, while at the same time alkaline phosphatase and urinary amino acids remained normal. In view of these findings, a diagnosis of idiopathic phosphaturia had been made, and treatment had been altered to include high phosphate feeding. In spite of this therapy, the patient died.

Microdissection of the nephron had shown that the proximal tubule was anatomically normal, but there were focal areas of increased translucency of a non-specific type, somewhat similar to that seen in acute tubular necrosis and in salt-losing states (Darmady, Griffiths, Mattingly, Spencer, Stranack & de Wardener, 1955; Darmady & Stranack, 1955). Perhaps the most striking finding was the presence of opaque deposits lying both in the epithelium and on the outer surface of the tubule (Plate IV, fig. Q). These changes, although present in the proximal tubules, were most marked in the distal tubule, and in places had apparently ruptured through the wall to cause small granulomas. The opaque nature of the material, with the obvious tissue reaction which occurred, made us believe that they corresponded to the areas of calcium deposition. The cause of the nephrocalcinosis was not clear, but it is possible that the previous high dosage of vitamin D might have played a part; however, it was interesting to note that the deposition of calcium could occur in all parts of the nephron.

[1] Porter, K. A. & Giles, M. M. (1956) *Arch. Dis. Childh.* **31**, 303—published after the above was set in type.—ED.

7. The Kidney in Potassium-Losing States

In 1955 Conn (1955a, 1955b) published an account of a new syndrome, associated with periodic severe muscular weakness, intermittent tetany, polyuria, polydipsia, and occasionally hypertension. Investigations showed that the victims of this syndrome had a severe hypokalaemia, with excessive loss of potassium in the urine and sweat, hypernatraemia, alkalosis, and increased urinary excretion of aldosterone, but with serum calcium and phosphate values within normal limits. The urinary excretion of 17-hydroxycorticoids and ketosteroids was also normal. The patients were also unable to produce acid urine and were resistant to pitressin.

Examination of kidneys of such patients by biopsy shows wide-spread vacuolation of the tubular epithelium (Schwartz & Relman, 1953; Conn, 1955a, 1955b), and this is also seen in conditions producing excessive potassium depletion (Williams & MacMahon, 1947; Relman & Schwartz, 1955), and in experimental animals on potassium-deficient diets (Schrader, Prickett & Salmon, 1937; Follis, Orent-Keiles & McCollum, 1942; Relman & Schwartz, 1955; Fourman, McCance & Parker, 1956).

Recently we have had the opportunity to examine the renal material from four cases showing potassium deficiency; these comprised one case of adrenal adenoma (Chalmers, Fitzgerald, James & Scarborough, 1956), one of adrenal carcinoma, and two of Cushing's syndrome. Our findings have been compared with four cases of Cushing's syndrome and two cases of multiple carcinomatosis without potassium depletion. In each case the renal material was removed at operation for adrenalectomy, and was therefore strictly comparable. The results of these findings are shortly to be published elsewhere.

In potassium-deficiency states there was intense vacuolation of the epithelium in both the proximal and distal tubule, most marked in the former (Plate IV, figs. R, S), but vacuolation was not found in the control cases. Such vacuolation is not dependent on an increase in the level of circulating adrenocortical steroids, since similar changes can be produced by potassium depletion alone.

ACKNOWLEDGEMENTS

We would like to thank all those who have sent us kidneys for investigation by microdissection, and particularly the donors named in Table I. We are grateful to Professor Pulvertaft for the phase-contrast photographs shown in Plate III, figs. C, E, G and H. Finally, we would like to express our thanks to the Medical Research Council for an expenses grant which has made this work possible.

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RENAL AMINOACIDURIA

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1 Cystinuria
2 Galactosaemia
3 Hartnup disease
4 Concluding remarks
References

Most of the free amino acids normally present in the glomerular filtrate are very efficiently reabsorbed by the renal tubules, and only very small quantities appear in the urine. The reabsorptive processes involved are not understood, but they are probably rather complex and not the same for all amino acids. Furthermore the tubular reabsorptive capacity is not unlimited, and this also will no doubt be found to vary for different amino acids or groups of amino acids. In consequence an abnormal urinary excretion of one or more amino acids may arise in at least two rather different types of situation. There may be some defect in intermediary metabolism, leading to an increase in plasma levels of one or more amino acids to such a degree that the normal renal tubular reabsorptive mechanism is unable to deal with them adequately, and they therefore pass in increased quantities into the urine. This is usually referred to as " overflow " aminoaciduria. On the other hand there may be some defect in the tubular reabsorptive mechanism such that, even at normal plasma concentrations, and hence normal concentrations in the glomerular filtrate, inefficient reabsorption of one or more amino acids takes place, and consequently abnormal quantities appear in the urine. Such a situation is called " renal aminoaciduria ".

With the wide-spread application during the last decade of chromatographic methods of separation to the study of the urinary and plasma amino acids, considerable advances have been made in this field. Much of this work has already been reviewed in the *British Medical Bulletin* (Dent & Walshe, 1954), and other reviews dealing with different aspects of the work have also appeared (Dent, 1954a; Harris, 1956). Attention here will therefore be directed only to some of the more recent contributions.

The three conditions which will be discussed are cystinuria, galactosaemia, and " Hartnup disease ". Each of these represents a form of renal aminoaciduria which is genetically determined. However, the series of causal relations giving rise to the aminoaciduria, and the detailed character of the aminoaciduria itself, differ greatly from one to another. In consequence, a consideration of these three disorders illustrates well the manner in which studies of this kind may hope to throw light on renal physiology and pathology.

1. Cystinuria

It has now been clearly established that in classical cystinuria, with recurrent cystine calculus formation, the urine contains not only grossly abnormal amounts of cystine, but also of the basic amino acids—lysine, arginine and ornithine (Dent & Rose, 1951; Stein, 1951). The quantities involved are remarkable. On the average, 0.75 g. of cystine, 1.8 g. of lysine, 0.8 g. of arginine, and 0.4 g. of ornithine are excreted daily. This is continuous and apparently persists throughout life. In urine with a pH between 5 and 7, cystine is soluble only to the extent of 0.3–0.4 g./l. (Dent & Senior, 1955); so that in patients excreting 0.5–1 g./day the concentration will frequently reach saturation level. Consequently the cystine tends to come out of solution, and this is presumably the prime factor in stone formation. Lysine, arginine, and ornithine, on the other hand, are freely soluble and therefore do not become incorporated in the stones. Stone formation probably occurs mainly during the night when the urine passed is most concentrated.

In 1949 Dent suggested that the aminoaciduria in cystinuria was renal in character and not, as had been previously thought, due to a block in intermediary metabolism. Extensive evidence has now accumulated in favour of this view. It has been shown that the plasma levels of the four amino acids concerned are not greater than normal, and indeed in most cases appear

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to be slightly lower (Harris, Fowler & Warren, 1952; Dent, Heathcote & Joron, 1954; Dent, Senior & Walshe, 1954; Stein & Moore, 1954). Furthermore the renal clearances of these four amino acids are grossly elevated, while those for the other amino acids are normal (Dent, Senior & Walshe, 1954; Arrow & Westall, personal communication, 1956). Finally a variety of metabolic experiments involving the feeding of cystine, methionine, and cysteine have failed to reveal any material difference between the metabolism of these amino acids in normal persons and those with cystinuria (Dent, Heathcote & Joron, 1954; Dent, Senior & Walshe, 1954), and in each case the results can be readily understood on the renal hypothesis.

The renal hypothesis implies that in normal individuals the tubular reabsorption of cystine, lysine, arginine and ornithine has at least one step which is common to and specific for these four amino acids, and that in cystinuria this process is in some way defective. If this is so, one might expect that, if one artificially elevated the plasma concentration of one of these amino acids to very high levels in a normal person, then—as a result of overloading the reabsorptive mechanism—an increased excretion of the other three amino acids should occur. This phenomenon has recently been demonstrated by Robson & Rose (1957) who infused lysine intravenously.

It has been known for many years that more than one patient with cystine stone formation may not infrequently be found in the same family. It is now clear that, even among the apparently healthy relatives of such patients, many individuals may be found with an unusual excretion of cystine and lysine and occasionally also of arginine and ornithine (Harris & Warren, 1953; Harris, Mittwoch, Robson & Warren, 1955a). Indeed all excretion values may occur among such healthy relatives, ranging from the very low amounts found in a random sample of normal subjects to the very large quantities encountered in those who form stones. Among such individuals there is a close correlation between the excretion of cystine and lysine. On the other hand excretion of arginine and possibly also ornithine is found to be elevated only when the cystine and lysine excretions are relatively high (Harris *et al.* 1955a). This suggests that there occurs a wide range of partial failure of tubular reabsorption of these amino acids and that, when the disturbance is not very severe, arginine and ornithine are preferentially reabsorbed to cystine and lysine.

Genetical analysis (Harris & Warren, 1953; Harris, Mittwoch, Robson & Warren, 1955b; Harris & Robson, 1955) has revealed a rather complex situation. More than one different abnormal gene may in homozygotes lead to a severe failure in the tubular reabsorption of the four amino acids. However, only in certain cases do the heterozygotes show any peculiarity, and here there is in most cases only a moderate degree of tubular dysfunction, and cystine and lysine are the only amino acids present in the urine in abnormal amounts.

2. Galactosaemia

In galactosaemia the patients are unable to metabolize galactose completely. Infants with this condition, when fed on an ordinary milk diet, show a high blood galactose level and excrete galactose in large amounts in their urine. Under these conditions they fail to thrive, their livers enlarge and eventually become cirrhotic, they develop cataract, and they frequently die in early life. If, however, such a child is fed on a galactose-free diet, the blood galactose level falls, and its physical condition rapidly improves.

Holzel, Komrower & Wilson (1952) discovered that the urine of such infants contained abnormal amounts of amino acids. Paper chromatography indicates that there is a considerable increase in the excretion of serine, glycine, threonine and alanine, and a moderate increase in the excretion of glutamine, valine, leucine and tyrosine (Holzel *et al.* 1952; Bickel & Hickmans, 1952; Cusworth, Dent & Flynn, 1955). So far, no quantitative data have been published.

At first sight the occurrence of aminoaciduria in a condition known to be associated with severe liver damage would suggest that the aminoaciduria was of the " overflow " type. However, it has now been established that in most cases it is purely renal in origin. The plasma level of α-amino-nitrogen is within normal limits (Komrower, 1953; Hsia, Hsia, Green, Kay & Gellis, 1954; Cusworth *et al.* 1955), and the α-amino-nitrogen clearance is raised significantly (Cusworth *et al.* 1955).

The fundamental defect in carbohydrate metabolism in galactosaemia appears to lie in an inability to carry out the conversion of galactose-1-phosphate to glucose-1-phosphate (Schwartz, Goldberg, Komrower & Holzel, 1955). It has been demonstrated that there is a complete or almost complete absence from erythrocytes, of the enzyme which catalyses the reaction of galactose-1-phosphate with uridine diphosphoglucose to give glucose-1-phosphate and uridine diphosphogalactose (Kalckar, Anderson & Isselbacher, 1956). This leads in the presence of galactose to an accumulation of galactose-1-phosphate. It is thought probable that the same enzyme deficiency occurs in all the tissues where the conversion of galactose-1-phosphate to glucose-1-phosphate takes place. It is a plausible hypothesis that the diverse series of disorders, including the renal tubule damage, which occur in this condition are due to some " toxic " effect of an intracellular accumulation of galactose-1-phosphate.

Some support for the concept that a build-up of material toxic to the renal tubule is responsible for the aminoaciduria, is provided by a consideration of the time relations in the appearance of the aminoaciduria following the withdrawal and subsequent re-addition of galactose to the diet. This has been studied by Cusworth *et al.* (1955). If galactose is completely removed from the diet the aminoaciduria will disappear and, if subsequently galactose feeding is recommenced, the aminoaciduria develops again. However, whereas the galactosaemia and galactosuria appear and cease almost immediately on commencing or stopping galactose feeding, the aminoaciduria takes some days to become apparent and some days before it ceases again.

3. Hartnup Disease

This is a recently discovered condition which has been described under the title " Hereditary pellagra-like skin rash with temporary cerebellar ataxia, constant renal aminoaciduria and other bizarre biochemical features " (Baron, Dent, Harris, Hart & Jepson, 1956). It has also been referred to briefly in various publications as Hart's syndrome and Hartnup syndrome (Bligh, 1953; Dent, 1954a; Bonetti & Dent, 1954; Dent, 1954b; Jepson, 1955; Evered, 1956).

This curious disorder was first found in four out of a family of eight brothers and sisters. The parents were first cousins and unaffected, and it is therefore highly probable that the

disease is inherited as a mendelian recessive character. Clinically the older of the affected sibs showed a severe pellagra-like skin rash and a peculiar neurological disturbance with mainly cerebellar features. The clinical state was, however, extremely variable, and severe attacks alternated over the years with almost complete remissions.

All the affected individuals have a gross renal aminoaciduria; this is constant and continuous and does not reflect variations in the clinical picture. The pattern of amino-acid excretion is quite different from any which has been encountered in other diseases and appears to be very specific for this condition. Alanine, serine, asparagine, glutamine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, and histidine are excreted in greatly increased amounts compared with the normal. The excretion of cystine, lysine and glycine is only moderately raised, and there is no abnormal excretion of proline, hydroxyproline, methionine, arginine or taurine. The amino-acid concentrations in the plasma lie within normal limits, and the renal clearances of those amino acids excreted in excess are grossly elevated (Evered, 1956). There is little doubt that there exists a severe and highly specific disturbance in the renal tubule reabsorption of amino acids.

The other outstanding biochemical finding in this disease is the occurrence of abnormal amounts of indican, indolyl acetic acid and another indolic compound in the urine (Jepson, 1955).

So far it has not been possible to formulate any satisfactory unifying hypothesis which would adequately account for the diverse clinical and biochemical features of this syndrome. It may be that one is dealing with some primary disorder of tryptophan metabolism, and that the renal tubular dysfunction is secondary to this. On the other hand there could be a primary disorder of amino-acid transport mechanisms, of which the renal tubular disorder is but one facet, and the disturbance in indole metabolism a secondary consequence.

4. Concluding Remarks

The three conditions which have been discussed here are each examples of genetically determined renal aminoacidurias. Nevertheless they are very diverse in character. In cystinuria and Hartnup disease the renal tubular dysfunction is constant and probably lifelong. In galactosaemia it is readily reversible by altering the carbohydrate content of the diet. In each case the pattern of amino-acid excretion, and hence the character of the tubular lesion, is characteristic and quite different from that found in the other conditions. Evidently the tubular reabsorptive processes are complex and may be disturbed at many different points, and in a variety of different ways. It seems reasonable to expect that, as the causal sequence of events in these and other similar conditions is unravelled, much new light will be thrown not only on the pathology but also on the physiology of the renal function. Gene-controlled disorders are of special interest from this point of view because they provide for study subjects with highly specific lesions, of a type which can rarely be produced by ordinary experimental procedures.

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ROLE OF THE KIDNEY IN EXPERIMENTAL HYPERTENSION

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2 Renal hypertension
  a Early phase
  b Late phase
3 Renoprival hypertension
4 Relationship of renal and renoprival hypertension
5 The role of renin
6 Nature of the extra-renal pressor mechanism
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1. Definitions

Hypertension can be induced in animals by various manipulations of the kidney such as partial constriction of the renal artery, compression of the kidney by a broad ligature, or by surrounding the organ with a cellophan capsule. High blood pressure produced in this manner will be termed renal hypertension. When the renal artery is partially constricted by a clamp (as in a dog) or by a clip (as in a rabbit or rat) the operation will be referred to as " clamping " or " clipping " and the kidney as the " clamped " or " clipped " kidney. If the opposite kidney is not removed and is not manipulated in any way it will be known as the " untouched " kidney. Hypertension can also be produced by removal of both kidneys; this will be referred to as " renoprival " hypertension (Ledingham, 1951). The aim of this paper is to discuss the mechanisms of renal and of renoprival hypertension and to determine the relationship between them.

2. Renal Hypertension

Goldblatt, Lynch, Hanzal & Summerville (1934) showed that in dogs partial constriction of the renal artery and removal of the opposite kidney produce persistent hypertension. In the years following this experiment it was considered that the blood pressure is elevated and maintained at a higher level by the secretion of renin from the kidney. More recently, however, it has become apparent that there are two phases in renal hypertension: an early phase, in which a renal pressor substance may be secreted by the kidney, and a late phase, in which it appears that the high blood pressure is maintained by some mechanism outside the kidney.

a. Early Phase

Pickering (1945) was the first to show that there may be two phases in renal hypertension. He demonstrated a fall of blood pressure to normal following removal of a single clipped kidney in rabbits with hypertension of one week's duration, but observed that the same operation caused no fall in blood pressure in rabbits with hypertension of eight weeks' duration. Haynes & Dexter (1947) reported the presence of renin in the blood of dogs within 24 hours of renal artery constriction; the concentration rose to a maximum after a week. After three months' hypertension, however, they were unable to demonstrate any renin. Kahn, Skeggs & Shumway (1950) found angiotonin in excess in the blood of dogs in the early phase of renal hypertension. From these experiments it appears that renin and angiotonin are in the circulation in the early phase of renal hypertension. It has been suggested that, since repeated injections render animals tachyphylactic, renin will not produce sustained hypertension. Blacket, Depoorter, Pickering, Sellers & Wilson (1950), however, were able to maintain persistent hypertension in rabbits for up to 18 days with a slow infusion of renin. They demonstrated that, when the infusion is stopped, the blood pressure falls to normal at the same rate as when the clipped kidney is removed in rabbits with hypertension of short duration. On the other hand, they found that during infusion the pressor response of a single injection of renin is greatly reduced. This work shows that renin can maintain sustained hypertension. However, Flasher & Drury (1950) showed that rabbits with early renal hypertension can be rendered tachyphylactic to renin without reducing their blood pressure. They also demonstrated that the pressor response to a single injection of renin is slightly increased in early renal hypertension, in contrast to the reduced response observed by Blacket *et al.* (1950) in rabbits undergoing renin infusion. Floyer (1955), working with rats, compared the rate of rise of blood pressure after total nephrectomy and after renal artery constriction of differing degrees of severity. He argued that renal artery constriction might cause hypertension either by secretion of a renal pressor substance or by inhibition of a function of the kidney concerned in maintaining normal blood pressure. In the latter event, it would be expected that the rise of blood pressure after renal artery constriction would be at the same rate as the rise observed after total nephrectomy, or at a lower rate than this. If, however, the blood pressure after renal artery constriction rose more rapidly than after total nephrectomy it would be probable that a renal pressor substance was responsible.

In the rats in which both kidneys were removed, the blood pressure remained steady for three days and then rose steeply. The rats in which one renal artery was constricted and the other kidney removed could be divided into three groups: (i) In a few animals the blood pressure rose rapidly during the first three days after operation, but these all died within five days and were found to have extensive renal infarcts. (ii) In most of the rats which survived more than five days the blood pressure rose more slowly than in the group in which both kidneys were removed. (iii) In a few animals the blood pressure rose somewhat more rapidly than in the nephrectomized group for the first three days, but after this rose at the same rate or more slowly. This experiment therefore gave distinct evidence of the presence of a renal pressor factor only during the first three days after clipping or when severe renal infarction had occurred. Although the existence of such a factor could not be excluded in the animals in which the blood pressure after clipping rose more slowly than after nephrectomy, it was considered that the slow rise is more suggestive of a mechanism like that of renoprival hypertension than of a renal pressor substance.

Floyer also showed that after renal artery constriction, even though no rise in blood pressure has occurred, rats show a

more rapid pressor response to nephrectomy. In animals in which no rise in blood pressure had occurred five days after constriction of one renal artery and removal of the opposite kidney, the clipped kidney was excised. In these animals the blood pressure rose more rapidly than in rats in which both kidneys were removed, one five days after the other, without previous renal artery constriction. He suggested that renal artery constriction might set off the same mechanism as raises the blood pressure after total nephrectomy; and that in the animals with previous renal artery constriction this mechanism had been started before the second kidney was removed.

It is probable that species difference accounts for some of the conflicting results obtained by different workers. The evidence is, however, suggestive that there is an early phase after renal artery constriction in the rabbit and in the dog in which a renal pressor substance elevates the blood pressure. This substance may or may not be renin. In the rat, the evidence suggests that a renal phase may only sometimes occur. A good deal more work is necessary before the mechanism of the early phase of experimental hypertension can be understood.

b. Late Phase

In 1945 Pickering showed that removal of a single clipped kidney in rabbits with renal hypertension of eight weeks' duration does not lower the blood pressure, but that the hypertension persists until a short time before the animals die. Daniel, Pritchard & McQuaid (1954a) have confirmed this observation in rabbits. Grollman (1946) showed that, in rats, chronic hypertension persists after total nephrectomy, and Floyer (1951) confirmed this finding. Floyer (1954) also showed that, in rats, when hypertension has been present for as little as two weeks, no fall occurs after total nephrectomy. Kolff & Page (1955b) demonstrated that, in dogs, chronic renal hypertension persists after total nephrectomy. These observations indicate that in the dog, in the rat, and in the rabbit, some extra-renal mechanism must be responsible for maintaining the elevated blood pressure in the later stages of hypertension. One explanation of this might be that irreversible narrowing occurs in the arterioles after a few weeks' hypertension, but microscopic evidence of this has never been demonstrated. Another explanation is that the baroceptor mechanism might become "reset" for a high level and that the sympathetic nervous system might maintain the raised blood pressure even though the renal pressor factor has ceased operating. It is obvious that, for hypertension to occur at all, the baroceptor mechanism must be reset for a higher level, but this does not necessarily imply that it is this mechanism alone which is responsible for maintaining the high blood pressure. Byrom & Dodson (1949) showed that in rats with chronic renal hypertension (12 weeks) removal of the clip from the renal artery restores the blood pressure to normal within 12 hours. This observation indicates that even in chronic hypertension, i.e., in the phase at which nephrectomy fails to abolish hypertension, removal of the renal artery constriction nevertheless restores the blood pressure to normal. This finding excludes the possibility that persistence of hypertension after nephrectomy might be due to permanent changes in the blood vessels, or that the blood pressure might be maintained by the sympathetic nervous system independently of the kidney. These results have been confirmed by Blacket & Sellers (1951) who showed that the blood pressure of rabbits with chronic hypertension fell to normal after removal of the clip, although in some animals the fall was more gradual and took several days.

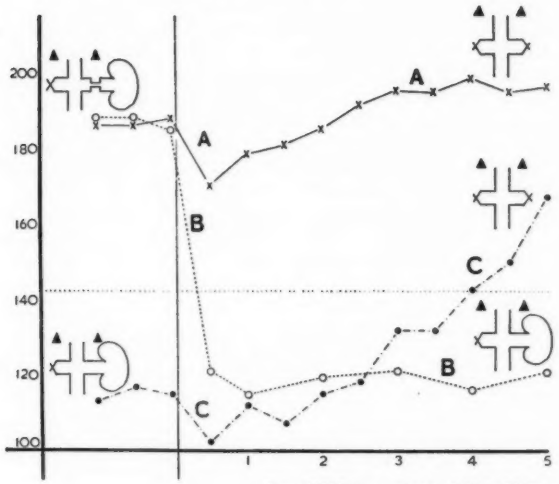
Daniel, Pritchard & McQuaid (1954b) observed a fall to normal after removing the clip in only four out of twelve rabbits with chronic renal hypertension. They demonstrated, however, that even after the removal of the clip the renal artery remained flattened or kinked in most of their animals. Floyer (1951) repeated Wilson & Byrom's (1939) work and made rats hypertensive by constriction of one renal artery without removal of the opposite kidney. In spite of the high blood pressure most of the animals remained well for up to 20–50 weeks. Extensive damage, due to hypertensive vascular lesions, as described by Wilson & Byrom (1941), was found in the untouched kidney, but the clipped kidney remained structurally normal. Removal of the clip caused a considerable fall of blood pressure, although not to normal in all animals. Subsequent excision of the kidney from the renal artery of which the clip had been removed, leaving behind the damaged untouched kidney, resulted in persistent hypertension; excision of the damaged untouched kidney, leaving the structurally normal kidney, restored the blood pressure to normal in all instances. Excision of abnormal kidney tissue and restoration of normal circulation to a normal kidney will restore normal blood pressure after 50 weeks' hypertension; clearly, renal hypertension in the late stage is not maintained by a mechanism independent of renal control.

We must now consider the striking difference in the response of animals in the late phase of hypertension to nephrectomy and to removal of the clip. This is illustrated in fig. 1 (from Floyer, 1955) which sums up the experiments described above. Curve A is the mean blood pressure of a group of rats with hypertension of several weeks' duration produced by renal artery constriction and removal of the opposite kidney; it can be seen that the hypertension persists after nephrectomy. In a group of rats with hypertension of similar duration and degree, removal of the clip restores the blood pressure to normal (curve B). We can explain these results by postulating that one function of a normal kidney is to maintain normal blood pressure and that in the late phase of hypertension this function is lost. Restoration of normal circulation to the kidney restores this function, and the kidney brings the blood pressure back to normal. This hypothesis assumes that some mechanism must exist outside the kidney which tends to raise the blood pressure but which is normally held in check by the kidney; this mechanism will be called the "extra-renal pressor mechanism". If such a mechanism exists, one would expect that the blood pressure would rise in normal animals after removal of both kidneys. As stated above, this does happen, and this form of hypertension will now be discussed.

3. Renoprival Hypertension

Grollman & Rule (1943) were able to demonstrate that hypertension occurs when both kidneys are removed from one member of a pair of parabiotic rats. This was confirmed by Ledingham (1951). Grollman, Muirhead & Vanatta (1949) demonstrated that dogs can be kept alive for several months after total nephrectomy by dialysis and that these animals have persistent hypertension and develop hypertensive vascular lesions. Kolff & Page (1954b) also produced hypertension after total nephrectomy in dogs and showed that

FIG. 1. MEAN BLOOD-PRESSURE CURVES OF THREE GROUPS OF RATS



Floyer (1955) by permission of Clinical Science

Abscissae: days after operation
Ordinates: blood pressure (mm. Hg)

Curve A: rats with experimental renal hypertension subjected to total nephrectomy

Curve B: rats with experimental renal hypertension, in which clip was removed

Curve C: normal rats subjected to total nephrectomy

Dotted horizontal line represents upper limit of normal blood pressure.

the blood pressure can be restored to normal when blood from the hypertensive animal is perfused through a normal kidney. Floyer (1951, 1955) and Kolff & Page (1954a) demonstrated hypertension after total nephrectomy in rats. Curve C (from Floyer, 1955) in the figure is the mean blood pressure of a group of rats after total nephrectomy. Grollman *et al.* (1949) showed that the presence of the kidney in the body, even though not excreting, prevents the development of hypertension; dogs in which one kidney is removed and the other ureter is implanted into the inferior vena cava, so that all urine returns into the blood stream, do not develop hypertension. This was confirmed by Kolff, Page & Corcoran (1954). Floyer (1955) showed that rats in which one kidney is removed and the other ureter implanted into the vena cava do not develop hypertension unless hydronephrosis occurs in the remaining kidney. All these authors agree that renoprival hypertension is due to the absence of some function of the kidney, unconnected with excretion, which maintains normal blood pressure. Leonards & Heisler (1951, 1952) consider that renoprival hypertension is due solely to water overload; they were unable to induce hypertension in dogs after total nephrectomy unless the animals were over-hydrated. Orbison, Christian & Peters (1952) showed that hypertension can be brought on very rapidly after total nephrectomy by an injection of normal saline. Kolff & Page (1954a) and Kolff *et al.* (1954) in a careful study have demonstrated that, while over-hydration in both rats and in dogs accelerates the rate of development, renoprival hypertension will nevertheless occur in the absence of over-hydration. Moreover, they were unable to produce hypertension by over-hydration in dogs in which one kidney had been removed and the opposite ureter implanted into the vena cava. Ledingham (1954) and Floyer (unpublished observations) have found that renoprival hypertension occurs in rats even though no salt is given after nephrectomy, but that saline accelerates the rate at which hypertension develops.

The conclusion appears to be that the normal kidney, by some function unconnected with excretion, maintains normal blood pressure by inhibiting the extra-renal pressor mechanism which causes hypertension after total nephrectomy. Over-hydration accelerates this type of hypertension, but is not essential for it.

4. Relationship of Renal and Renoprival Hypertension

It has been shown that in the late stage of experimental renal hypertension the mechanism by which the high blood pressure is maintained must be extra-renal, since removal of the kidneys does not affect the hypertension. In addition, an extra-renal pressor mechanism has been shown to operate when both kidneys are removed in a normal animal. It is pertinent to ask whether these two mechanisms are the same and whether in the late stage of hypertension the kidney fails to perform the function of maintaining normal blood pressure.

The evidence in favour of this unitary hypothesis is strong, but not conclusive: both types of hypertension are abolished when a normal kidney with normal circulation is introduced into the circulation, both forms are abolished by adrenalectomy and restored by giving salt (Floyer, 1951), similar differences in electrolyte composition of the fluid compartments have been demonstrated (Ledingham, 1953, 1954). There is, however, one observation which appears at first sight definitely to exclude this unitary hypothesis, namely that it is possible to induce persistent hypertension by renal artery constriction without removal of the opposite kidney. Removal of one kidney does not cause hypertension; it would not be expected that renal artery constriction on one side would do so if this operation does no more than prevent one kidney from maintaining normal blood pressure. In the rabbit, and in the dog, renal artery constriction without removal of the opposite kidney produces only a transient rise of blood pressure, possibly due to the secretion of a renal pressor substance; usually it is not possible to maintain persistent hypertension. However, in the rat, and sometimes in the rabbit and in the dog, persistent hypertension can be produced in the presence of an apparently normal untouched kidney. It is true that the untouched kidney may suffer vascular damage, but this does not usually occur until much later. The explanation could be: (i) that the early renal phase lasts much longer in animals with two kidneys than in animals with one; (ii) that the clipped kidney exerts some influence on the untouched kidney and prevents the latter from exercising its function of maintaining normal blood pressure.

The obvious experiment to decide this is to remove both kidneys simultaneously. Floyer (1954) showed that, in rats with a clip on one renal artery, if hypertension has been present for more than two weeks, bilateral nephrectomy does not lower the blood pressure, indicating that the early or renal stage is over after two weeks and that an extra-renal mechanism is operating. In contrast, removal of the clipped kidney alone in rats with hypertension of similar duration

restores the blood pressure to normal in a few hours. These observations support the hypothesis that the clipped kidney exerts some influence on the untouched kidney and prevents the latter from exercising the function of maintaining normal blood pressure. This could be done either by a humoral factor or by the nervous system; it has been shown that denervation of both kidneys does not prevent the development of hypertension (Floyer, 1954). This suggests that the clipped kidney secretes a substance which does not necessarily have a generalized vasoconstrictor effect but which prevents the opposite kidney from maintaining normal blood pressure. No such substance has yet been isolated, but Gordon & Flasher (1951) have demonstrated that a rabbit kidney from which the circulation has been completely cut off makes a substance which causes a vasoconstrictor response in the opposite kidney but which has no effect upon the rest of the body. This work has not yet been confirmed but, if true, it might explain why, in some animals, renal artery constriction on one side produces in the opposite kidney a change in function similar to that produced in the clipped kidney.

5. The Role of Renin

Although it appears from the work discussed above that renin plays a part only in the early stage of hypertension, some work by Wakerlin, Bird, Brennan, Frank, Kremen, Kaperman & Skom (1953) must now be considered. They have demonstrated that, if hog renin is injected repeatedly into dogs, antibodies against renin are formed. Dogs treated in this manner fail to develop hypertension following renal artery constriction; in dogs with existing hypertension, even in the late stage, the blood pressure is restored to normal. Moreover, if dogs in the late stage of hypertension are given an injection of serum rich in antibodies against renin (" anti-renin "), the blood pressure falls to normal for a short time. Control animals injected with antibodies against other proteins do not show a similar fall in blood pressure. In spite of the failure to find significant quantities of renin in the blood in the late stage of renal hypertension and in spite of the failure of nephrectomy to restore the blood pressure to normal, this work supports the possibility that renin plays some part even in the later stage. Kolff & Page (1955b) demonstrated that in dogs an injection of anti-renin has no effect upon hypertension after removal of both kidneys. This excludes the possibility that renin might persist in the circulation and might be responsible for hypertension after nephrectomy. It does not explain, however, why anti-renin reduces the blood pressure before nephrectomy; if renin is the cause of the hypertension it would be surprising for the mechanism to change suddenly after nephrectomy without affecting the blood pressure. Kolff & Page (1955a) have suggested that, although in the late stage of renal hypertension renin might not maintain hypertension by its peripheral vasoconstrictor effect, it might nevertheless have a local effect on the kidney and inhibit the function concerned with maintaining normal blood pressure. This would explain why anti-renin, by releasing the kidney temporarily from the effect of renin, produces a fall in blood pressure; it is as if anti-renin removes temporarily the renal artery constriction. It is possible that renin might be the substance by which the clipped kidney influences the untouched kidney; renin might not be present in sufficient quantities to cause a direct pressor effect, but even in small amounts might have a functional effect on the opposite kidney. This new suggestion of the role of renin in hypertension must be investigated further.

6. Nature of the Extra-Renal Pressor Mechanism

The final question concerns the nature of the extra-renal pressor mechanism and the manner by which the kidney maintains normal blood pressure. The tone, the reactivity, and possibly the actual size of the arteriolar smooth muscle cells are influenced by many known factors, and probably by many more unknown. Smooth muscle is known to be sensitive to changes in electrolyte concentration; Ledingham in this number of the Bulletin, p. 33, discusses the changes in intracellular electrolytes demonstrated in hypertension. It is possible that the kidney, by some metabolic function, maintains intracellular electrolyte concentration and that failure to perform this function results in a change in the intracellular electrolytes of the smooth muscle cells of arterioles which leads to hypertension. Whatever may be the explanation, it is obvious that the problem is complex, and the above analysis may well be proved much too simple. Much more work must be done to test the various hypotheses discussed in this article.

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DISTURBANCES IN WATER AND ELECTROLYTE METABOLISM IN EXPERIMENTAL HYPERTENSION

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1 The intake of sodium and potassium in relation to experimental hypertension
2 Changes in the distribution of body-water and electrolytes in experimental hypertension
  a Total body content of water and electrolytes
  b Water and electrolytes in the extracellular compartment
  c Water and electrolytes in the intracellular compartment
3 Renal handling of water and electrolytes in experimental hypertension
4 Conclusions
References

The close relationship between the metabolism of water and electrolytes and hypertension occurring in man and experimental animals has been demonstrated only in the last decade. However, over half a century ago, low-sodium diets were being advocated in the treatment of hypertension in man. During the 1930's the experiments of Goldblatt and his collaborators and of other workers in this field directed attention to the possibility of a renal pressor substance as the cause of hypertension. Attempts to establish the renin-angiotonin system as the sole basis for experimental renal hypertension have failed up to the present time, and evidence is accumulating that renal hypertension may be due to some extra-renal pressor mechanism brought into action through the depression of some normally existing, but at present unknown, renal function. This consideration is more fully dealt with in the article on renal and extra-renal factors in renal hypertension.[1] In the study of the nature of this extra-renal pressor mechanism operating in experimental renal hypertension and in hypertension occurring after total nephrectomy (hereinafter referred to as renoprival hypertension), the importance of the sodium (Na) intake and of the adrenals became apparent (Floyer, 1951; Ledingham, 1951). It was shown that the presence of the adrenals was necessary for the development of renal hypertension and that the hypertension might be partially maintained after adrenalectomy if a high intake of sodium chloride (NaCl) was provided (Floyer, 1951). Renoprival hypertension occurring in single and parabiotic rats is also dependent on the presence of the adrenals, and here again NaCl may partially substitute for the adrenals. These observations strongly suggested that both these forms of hypertension were closely linked with the adrenals and especially with the adrenal control of electrolyte metabolism. Furthermore it seemed possible that the extra-renal pressor mechanism might itself be some derangement in the control of electrolyte metabolism brought about by interfering with a specific function of, or by removing, the kidneys. In this article it is proposed to outline the observations which have been reported on the relationship between the intake of electrolytes and hypertension, the disturbances in the distribution of body-water and electrolytes actually observed in hypertension, and the alterations in the renal handling of water and electrolytes in hypertension. These observations have not so far provided an acceptable explanation of the exact role of electrolytes in the pathogenesis of hypertension, although there is little doubt that their role is of great and possibly primary importance.

[1] See Floyer, p. 29 of this number of the Bulletin.—Ed.

1. The Intake of Sodium and Potassium in Relation to Experimental Hypertension

As was mentioned above, a low Na intake in the diet was advocated many years ago for the treatment of hypertension in man (Ambard & Beaujard, 1905; Allen, 1920). However, the diets were difficult to construct and often resulted in no benefit. Allen points out that often they were insufficiently free from salt. Interest in this subject was again aroused in 1944 following Kempner's introduction of the rice diet in the treatment of hypertension (Kempner, 1944). The experimental parallel of this was soon forthcoming. Grollman & Harrison (1945) found that drastic reduction of Na in the diet of rats with renal hypertension rapidly depressed the level of blood pressure. This has been confirmed by others (Danford, Dieter, Christofferson & Herrin, 1950) and it has furthermore been shown that the same reduction of NaCl intake does not lower the level of blood pressure in normal animals (Frieden, Stamler, Hwang, Kuramoto & Katz, 1952; Grollman & Harrison, 1945).

The development of renoprival hypertension is facilitated by increasing the NaCl intake (Floyer, 1951; Ledingham, 1954a), and this action is not simply one of maintaining an adequate extracellular fluid (ECF) volume (Ledingham, 1954a). Hypertension resulting from the administration of the steroids cortisone and deoxycortone is independent of the NaCl intake in the case of cortisone, but strictly related to this intake in the case of deoxycortone (Knowlton, Loeb, Stoerk, White & Hefferman, 1952; Ledingham, 1954b). A high intake of NaCl may by itself result in moderate hypertension. This was first observed in chickens (Lenel, Katz & Rodbard, 1948), and later in rats (Sapirstein, Brandt & Drury, 1950). The hypertension so brought about is referred to as " salt hypertension ", and its production in rats seems dependent on the presence of the adrenals, although, once the hypertension has been produced, a considerable time elapses after adrenalectomy before the blood pressure falls. These observations are summarized in Table I.

Potassium (K) depletion has been reported to depress the blood pressure in normal rats and in rats with renal hypertension (Freed & Friedman, 1950; Friedman, Rosenman & Freed, 1951). Rats that are subjected to K-deficient diets are very weak and do not gain weight, and it is possible that the depression in blood pressure may be a non-specific effect; furthermore K deficiency is known to cause focal myocardial necrosis which may be contributory in lowering the blood pressure, although such lowering may apparently occur in the absence of visible myocardial damage. The depression in blood pressure brought about by K depletion has been found

TABLE I. EFFECT OF SODIUM ON THE BLOOD PRESSURE OF NORMAL AND EXPERIMENTAL HYPERTENSIVE RATS

	Normal	Experimental renal hypertension	Renoprival hypertension	Deoxycortone hypertension	Cortisone hypertension
Increasing Na intake	Blood pressure rises when intake greatly increased ("salt hypertension")	Hypertension slightly exacerbated	Hypertension exacerbated	Hypertension proportionately increased	Hypertension unaffected
Decreasing Na intake	Blood pressure unaffected	Hypertension depressed with extreme Na restriction	Hypertension depressed	Hypertension proportionately depressed	Hypertension unaffected

to be abolished by cortisone (Freed, Rosenman & Smith, 1954), but the significance of this observation is not at all clear.

Thus it will be seen that alterations in the intake of Na and K can not only influence many forms of experimental hypertension but may also alter the level of blood pressure in the normal animal. The action of these cations might be directly on the cardiovascular system or mediated at least partly through the adrenal cortex. That the action of Na cannot be entirely mediated through the adrenal cortex is evident from the fact that salt hypertension can be maintained, though not initiated, after adrenalectomy, and similarly that renal hypertension may be partially maintained after adrenalectomy by raising the intake of Na (Floyer, 1951). Further, renoprival hypertension can develop in the absence of the adrenals, providing a high intake of Na is permitted (Floyer, 1951). Our problem is to determine whether the influence of these cations on the blood pressure is an independent variable, or whether it is intimately bound up with the cause of various forms of hypertension—perhaps being the final common pathway in multiple chains of causation. The fact that the same degree of Na restriction produces a fall in the blood pressure of rats with renal hypertension but not of normotensive rats supports the latter of these two possibilities and suggests that some derangement of electrolyte metabolism may be the basis of renal hypertension. I shall return to this later in the discussion.

2. Changes in the Distribution of Body-Water and Electrolytes in Experimental Hypertension

From the observations in the previous section it is clear that changes in the intake of certain cations greatly influence various forms of experimental hypertension and it would appear logical to search for some alteration in the distribution of body-water and electrolytes peculiar to the hypertensive state. There are many approaches to this problem and many difficulties and fallacies to contend with. Investigations will include measurements of the water and electrolyte content of the whole body and of the extra- and intra-cellular compartments of the body.

a. Total Body Content of Water and Electrolytes

Greene & Sapirstein (1952) measured the total body Na, K and nitrogen (N) in rats with renal hypertension, and found an increased Na but normal N and K content. They suggested that Na was increased either because of segregation in bone or concentration within the cells. The possibility that total Na was raised because of expansion of the ECF volume was rejected. However, the technique used for producing hypertension is now known to be associated with expansion of the ECF volume, and the possibility that this was in part an explanation of their findings, cannot be excluded. No further work to confirm or refute these observations has been published.

b. Water and Electrolytes in the Extracellular Compartment

The ECF volume was reported to be elevated in experimental renal hypertension in both rats (Braun-Menendez & Covian, 1948) and dogs (Grollman & Shapiro, 1953). However, other studies suggested that the ECF volume, although raised in hypertension of short duration, fell to normal as the duration of hypertension increased (Ledingham, 1953), and it became clear that experimental renal hypertension could exist in the presence of a perfectly normal ECF volume.

The concentration of extracellular cations is normal in experimental renal hypertension (Ledingham, 1951) and thus it would appear that, if alterations in body-water and electrolytes are present in this form of hypertension, then the changes must exist in the intracellular compartment.

c. Water and Electrolytes in the Intracellular Compartment

The investigation of changes within the body-cells presents two difficult problems. Firstly, it is now recognized that different tissues vary widely not only in their content of cations but also in the freedom with which movements of cations in and out of the cell take place. Thus, under treatment with deoxycortone, Na goes freely into skeletal muscle but only slightly into cardiac muscle cells (Ledingham, 1954b). Hence we cannot make assumptions regarding the composition of electrolytes in one tissue from measurements in another. In considering the problem of hypertension, it would be logical to examine the various elements of the cardiovascular system, especially the cardiac muscle and the walls of the arterioles across which the greatest pressure drop occurs. Unfortunately the minute size of the latter precludes their use. Attempts have been made to study the composition of the walls of the larger elastic arteries, but their importance in determining the mean blood pressure is small, and furthermore their complex anatomical structure, with their walls comprising much elastic and collagen tissue, rather defies clear analysis in the present state of our knowledge. This will be referred to again later.

Secondly, there is the technical problem of determining the concentration of electrolytes within the cell. Simple measurements of the cation concentrations in whole tissues can be most misleading because of the ECF content: this applies particularly forcibly to Na whose concentration is so much

TABLE II. THE DISTRIBUTION OF SODIUM AND POTASSIUM IN PLASMA AND IN HEART AND SKELETAL MUSCLE IN RENAL HYPERTENSIVE AND NORMOTENSIVE RATS

			Hypertensive group	Normotensive group	Significance of difference of means
Plasma	Na	(m-equiv./l.)	144.2 ± 1.1	143.9 ± 0.6	
	K	(m-equiv./l.)	4.13 ± 0.11	4.18 ± 0.17	
Heart muscle	Total Na	(m-equiv./kg.)	33.5 ± 0.7	30.8 ± 0.4	*$t = 11.6$ $P < 0.01$
	Total K	(m-equiv./kg.)	89.8 ± 0.8	93.1 ± 0.6	$t = 3.15$ $P < 0.01$
	Na concentration in ICF	(m-equiv./l.)	22.2 ± 0.6	23.7 ± 0.6	$t = 1.78$ $0.05 < P < 0.1$
	K concentration in ICF	(m-equiv./l.)	147.2 ± 1.1	148.0 ± 1.0	
	Ratio $\frac{\text{ICF volume}}{\text{Solids}}$	(l./kg.)	2.45 ± 0.02	2.42 ± 0.02	
Skeletal muscle	Total Na	(m-equiv./kg.)	18.8 ± 0.6	18.8 ± 0.7	
	Total K	(m-equiv./kg.)	116.9 ± 0.5	116.1 ± 1.1	
	Na concentration in ICF	(m-equiv./l.)	12.2 ± 0.7	14.0 ± 0.7	$t = 1.83$ $0.05 < P < 0.1$
	K concentration in ICF	(m-equiv./l.)	172.5 ± 0.9	172.2 ± 0.9	
	Ratio $\frac{\text{ICF volume}}{\text{Solids}}$	(l./kg.)	2.70 ± 0.05	2.62 ± 0.04	

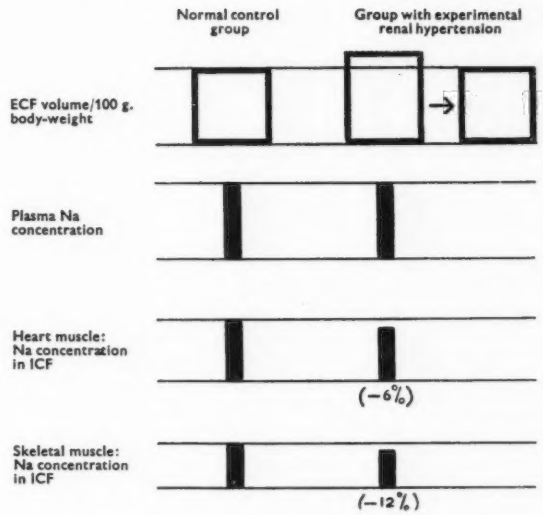
The mean values are given together with the standard errors.
The ratio of intracellular fluid volume to solids is considered to be a measure of the degree of intracellular hydration.
* t refers to Fisher's t-test and P to probability.

higher in extra- than in intra-cellular fluid. Moderate increases in interstitial fluid will cause very little change in the total water content of tissue but will greatly raise the Na content expressed in terms of wet or dry weight. This consideration most probably accounts for the earlier reports of raised Na concentration in heart and skeletal muscle in experimental hypertension (Eichelberger, 1943; Laramore & Grollman, 1950). Hence it is clear that an attempt must be made to define the extracellular compartment. The commonest method for doing this is to measure the chloride (Cl) content and make the assumption on very uncertain grounds that Cl lies entirely in the extracellular compartment under all experimental circumstances. Proof of this assumption is wanting, and interpretations based on it may well be erroneous.

Fewer objections can be raised against the use of a large relatively inert molecule, such as inulin, to define the extracellular space. It is proposed to outline the results of our experiences using the "inulin" technique to investigate the disturbances of body-water and of the cations, Na and K, in various forms of experimental hypertension (Ledingham, 1953, 1954a, 1954b).

The findings in renal hypertension induced in rats by applying a clip to one renal artery, the other kidney being either left in situ or removed, are shown in Table II and fig. 1. It will be seen that no changes take place in the ECF save that its volume is initially raised: after four weeks of hypertension the ECF volume returns to normal. This initial expansion of the ECF volume may or may not be an essential factor in the development of this form of hypertension, but it is clear that hypertension will persist in the presence of a perfectly normal

FIG. 1. DIAGRAMMATIC REPRESENTATION OF THE FINDINGS IN A GROUP OF RATS WITH EXPERIMENTAL RENAL HYPERTENSION, AND IN A NORMOTENSIVE CONTROL GROUP

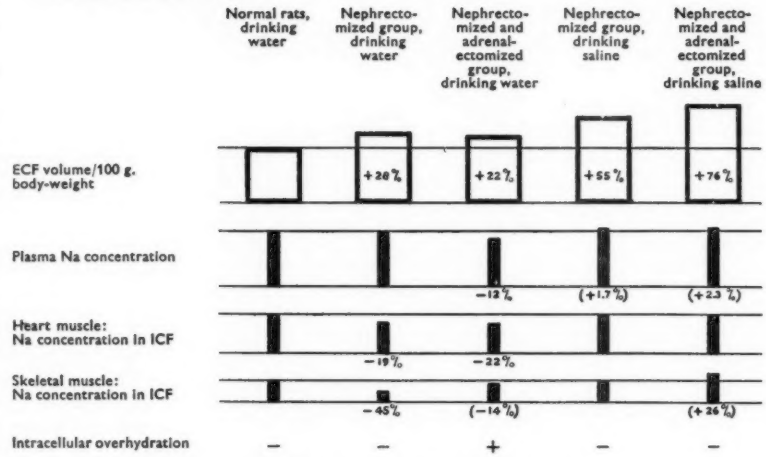


In this and in subsequent figures differences from the control group are expressed as a percentage of the latter, and are placed in brackets when not statistically significant.

ECF volume. There is some evidence that, after interfering with renal function in other ways, such as by removing one kidney and fixing the other below the skin, there may be transient expansion of the ECF volume unaccompanied by hypertension. This observation may well explain the persistent expansion of ECF volume observed by other workers, in rats with renal hypertension induced by applying constricting ligatures to the sole remaining kidney or to both kidneys, since such treatment might well be expected to interfere permanently with renal function.

In cardiac and skeletal muscle the only abnormality in renal hypertension was a decrease in the concentration of Na in the intracellular fluid (ICF). These changes were not marked enough to be statistically significant, but, in calculating the concentration of inulin in the interstitial fluid from the observed concentration in the plasma, what is now regarded as too great a correction was applied. If a more reasonable correction is applied these differences become significant; furthermore, any failure of complete equilibration of inulin between plasma and interstitial fluid would also tend to narrow the differences. Hence it is considered very probable that a true reduction in the concentration of Na in the ICF of heart and skeletal muscle does exist in renal hypertension. There is no doubt that these results completely rule out the possibility of an increase of intracellular Na in heart and skeletal muscle, an occurrence which had been thought likely by earlier workers.

FIG. 2. DIAGRAMMATIC REPRESENTATION OF THE FINDINGS IN A GROUP OF NORMAL RATS AND IN GROUPS OF RATS THREE DAYS AFTER NEPHRECTOMY WITH OR WITHOUT ACCOMPANYING ADRENALECTOMY



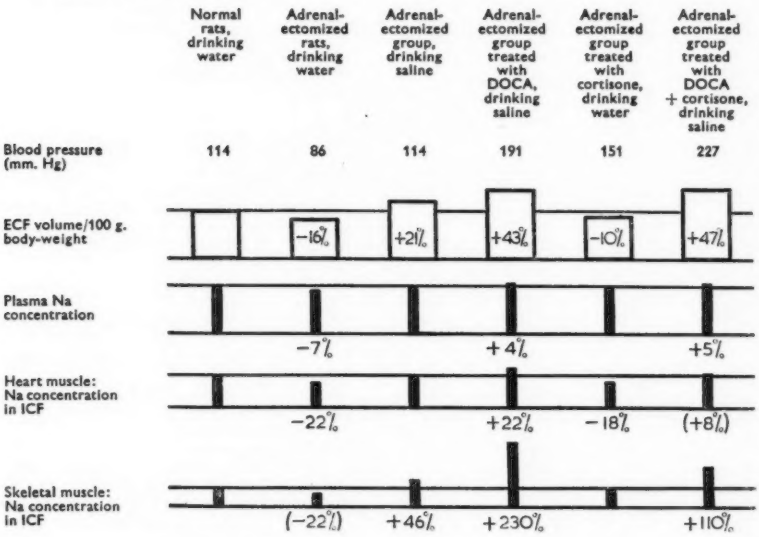
The groups drank either water or 0.5 % NaCl solution.

Studies similar to the above were carried out on rats three days after nephrectomy to investigate the changes in electrolyte distribution associated with renoprival hypertension. Half the rats drank water and the other half 0.5% NaCl solution. From a further group of rats both kidneys and adrenals were removed and the same observations made. The important results are shown diagrammatically in fig. 2. It will be seen that, when drinking water, the nephrectomized rats expanded their ECF volume yet maintained a normal concentration of extracellular Na, partly, at least, by withdrawing Na from within the cells of heart and skeletal muscle, whereas the rats with both kidneys and adrenals removed were unable to maintain the level of extracellular Na. In this latter group the concentration of Na fell also within the ICF, not as a result of Na passing out of the cell, but through water passing into the cell. Thus it would appear that the adrenals had the effect of withdrawing Na from the cells, so maintaining the level of extracellular Na. It is under these circumstances that renoprival hypertension develops, although in the short observation period (three days) of these experiments insufficient time had elapsed for it to do so. In the groups drinking 0.5% saline, the level of intracellular Na was maintained whether or not the adrenals were present. There was a tendency for the level of extracellular Na to rise, although this was not significant. Thus NaCl appeared to provide an adequate substitute for the adrenals in so far as the electrolyte distribution was concerned in these groups. The only group actually becoming hypertensive in the short duration of these experiments was the nephrectomized group drinking saline. Had the experiments been prolonged further, from previous experience the nephrectomized group drinking water and the group with both kidneys and adrenals removed drinking saline might also have been expected to develop hypertension. Again the results are not conclusive but it would appear that, in the groups actually or potentially hypertensive, there is either a fall in intracellular, or a tendency to rise in extracellular, Na concentration in heart muscle.

Studies have also been made on the changes in electrolyte and water distribution in adrenalectomized rats drinking either water or 1.5% saline and treated with deoxycortone and cortisone or a combination of both steroids. Some of the results are summarized in fig. 3. It will be noted that in adrenalectomized rats drinking water the concentration of Na in the ICF of heart and skeletal muscle fell. This can probably be attributed to the entry of water into the cellular compartment; and there was no evidence of movement of Na or of K into or out of the cells. Adrenalectomized rats treated with saline did not suffer any dilution of body-fluids and in fact Na entered skeletal muscle cells. This lability of Na within skeletal muscle cells was seen in many of the experimental groups in contrast to the relative constancy of Na in cardiac muscle cells. Cortisone treatment resulted in moderate hypertension accompanied by a fall in ECF volume, a normal level of plasma Na and a fall in Na concentration in the ICF of cardiac muscle. Treatment with saline and deoxycortone, or saline, deoxycortone and cortisone produced more marked hypertension and a rise in the plasma Na concentration. The concentration of Na in the ICF of heart muscle also rose slightly but much less than that in skeletal muscle. As mentioned above, the calculated figures for the concentration of Na in the ICF err on the high side, and it is considered that the concentration of Na in heart muscle in these two groups may really be within the normal range.

Reviewing the results in renal, renoprival and steroid hypertension, it is clear that there is no well-defined distribution of electrolytes characteristic of the hypertensive state. However,

FIG. 3. DIAGRAMMATIC REPRESENTATION OF THE FINDINGS IN A GROUP OF NORMAL RATS AND IN GROUPS OF ADRENALECTOMIZED RATS, SOME OF WHICH WERE TREATED WITH ADRENAL STEROIDS



The groups drank either water or 1.5 % NaCl solution.

there seems to be some evidence that hypertension is more likely to be present when there is a fall in the intracellular Na concentration in heart muscle relative to the extracellular, although much further work is required to establish this hypothesis. It may well be asked what significance, if any, would such a hypothesis, if true, have in relation to hypertension. No answer to this problem can be given in the present state of our knowledge. The changes might be the result of, or an associated phenomenon in, hypertension, rather than an aetiological factor in its production. Yet it does seem possible that changes in cation distribution within the affected muscle cells of the cardiovascular system might play some part in determining the force of contraction of the cardiac muscle fibre or the resting length of the smooth muscle of arteriolar wall.

It should be mentioned that much work has been reported on the changes in the distribution of water and electrolytes in the aortic wall of hypertensive rats. The findings are inconclusive and the results are extremely difficult to interpret in view of the complex anatomical structure of the aorta (Tobian & Binion, 1952, 1954a, 1954b).

3. Renal Handling of Water and Electrolytes in Experimental Hypertension

No detailed study has been made of the renal handling of water and electrolytes during the period of development of experimental renal hypertension. Once renal hypertension has become established there is evidence that the kidney responds normally to an intravenous Na load (Stamler, Hwang & Kuramoto, 1951). Thus in some dogs with renal hypertension the glomerular filtration rate (GFR) may be normal and in these there is a normal excretion of the Na load. In other hypertensive dogs where the GFR is lowered, there is also a lower rate of Na excretion but no change in the excretion expressed as a percentage of the filtered load. Recently another group of workers reported an accelerated excretion of a Na load imposed by gavage in rats with experimental renal hypertension and with hypertension resulting from the administration of deoxycortone and cortisone (Friedman, Hinke & Hardwick, 1955). The significance of this is obscure.

The interesting observation was made by Blake and his colleagues (Blake, Wegria, Ward & Frank, 1950) that minimal contraction of the renal artery, of insufficient degree to depress the GFR, was associated with a decrease in urine flow and Na excretion. These changes occurred at once and seem unlikely to have been mediated through any humoral mechanism. This would suggest that some intra-renal redistribution of blood might play a part in the tubular handling of Na excretion and might possibly be an important factor in the genesis of hypertension.

In a short review of this subject, attention must be drawn to much work (Grollman, Muirhead & Vanatta, 1949; Kolff & Page, 1954; Floyer, 1955) which supports the view that both experimental renal and renoprival hypertension are due to failure of some internal metabolic function of the kidney rather than of excretion. Thus hypertension will not develop if the ureters are implanted into the inferior vena cava, yet will do so if the kidneys are removed altogether; further, experimental renal hypertension will disappear if the clip is removed from the renal artery and at the same time the ureter is implanted into the inferior vena cava. Hence we would not necessarily expect to find any alteration in renal excretion of electrolytes either during the development or disappearance of hypertension brought about by any means. This paradox could be explained by a redistribution of electrolytes already present within the body.

4. Conclusions

The evidence, presented earlier, of the relationship between the intake of Na and the various forms of experimental hypertension makes it highly probable that disturbances in Na metabolism play an important role in hypertension. By this it is of course not meant that the intake of Na is the sole determinant of hypertension. In fact, in the intact animal, homeostatic mechanisms of electrolyte control permit rapid adaptations to all but extreme changes in intake of Na or other electrolytes. However, when these homeostatic mechanisms are upset, as, for instance, by interfering with the renal circulation, removing the kidneys or adrenals, or administering adrenal hormones, then the animal becomes extremely sensitive to changes in electrolyte intake. A clinical example of this is observed in patients who have undergone adrenalectomy for hypertension. In the present state of knowledge it is impossible to say just how such a disturbance in electrolyte metabolism might bring about hypertension. The possibilities

are many. The reported studies on the changes in distribution of body-water and electrolytes are no more than suggestive. Changes in cellular electrolyte content are known to be associated with alteration in membrane potential and these in turn may affect contractility either of the myocardium or of the smooth muscle of arteriolar wall. This could be the link with hypertension but at present it must remain purely hypothetical.

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THE TREATMENT OF HYPERTENSION IN PRIMARY RENAL DISEASE

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1 The importance of diagnosis
2 Indications for treatment and selection of cases
3 Results of treatment in renal hypertension
a Immediate effects of lowering the blood pressure
b Long-term results
4 Conclusions
References

During the past five years there has been a wide-spread increase in the use of drugs for the treatment of hypertension. In general the object of therapy has simply been to produce a lowering of the blood-pressure level on the grounds that high blood pressure may be undesirable and even dangerous. Thus in most patients the treatment has been directed towards hypertension as a disturbance of function, irrespective of the underlying disease process. Whilst most reported series chiefly refer to patients with essential hypertension, benign or malignant, diagnosis has been insufficiently stressed, so that it is often in doubt how many cases of chronic renal hypertension are included and whether the response to therapy in these patients is different from that in essential hypertension.

1. The Importance of Diagnosis

The recognition of hypertension due to unilateral renal disease or to phaeochromocytoma is an essential diagnostic preliminary which is now generally accepted and practised. There are, however, several reasons why a greater emphasis on the diagnosis of the underlying renal disease is of importance in hypertension. The evaluation of hypotensive drug therapy can be made only by comparison with the natural history of the disease in untreated cases. There can be little doubt that the natural history of renal hypertension differs in the great majority of patients from that of essential hypertension, since in the former the kidney is usually the site of long-standing renal disease with gross structural damage. Moreover the course and prognosis of the various types of renal hypertension, resulting for example from Type I nephritis, Type II nephritis (Ellis, 1942) or chronic pyelonephritis, may differ from one another as widely as does the underlying anatomical lesion. Most workers reporting the results of hypotensive drug therapy have recognized the importance of the natural history of the disease in selecting cases for treatment and have for this reason introduced some form of prognostic grouping. The grouping most commonly employed is based on the severity of retinal changes (Smirk, 1954). In practice this classifies patients with hypertension into those with no retinal haemorrhages or exudates, those who show such changes, and a third group with papilloedema—i.e. malignant hypertension. Such a classification is unreliable for two main reasons. Firstly, prognosis is variable and unpredictable in the first and second groups; only in the third, malignant hypertension, can a uniformly bad prognosis be expected in the absence of treatment, and it is for this reason that most writers have concerned themselves principally with the treatment of malignant hypertension. Secondly, the "eyeground classification" takes no account of aetiological differences, so that even the malignant hypertension group may include a variety of disease processes which, after reduction of the blood pressure, differ in their natural course and prognosis.

There are further reasons for discriminating between essential and renal hypertension when hypotensive drugs are used. In primary renal disease, where functional reserve is diminished by reduction in the number of effective nephrons, a fall in blood pressure may reduce blood flow and lead to deterioration in renal clearance. Hence hypotensive drug therapy may be harmful. On the other hand the relief of hypertensive heart failure which may be obtained by lowering the blood pressure often diminishes extra-renal nitrogen retention. This paper will describe observations on the effects of hypotensive therapy on renal function.

Lastly there is the consideration of the influence of hypertension on the pathological process in the kidney and in other organs. The observation of Wilson & Byrom (1939), that hypertension in the rat could give rise to arterial and glomerular lesions closely resembling those of malignant hypertension in man, provided a rational indication for hypotensive therapy as a prophylactic against such hypertensive vascular damage. Wilson & Byrom (1941) further showed that abolition of hypertension by excision of the clamped kidney was followed by remarkable healing of necrotizing arterial lesions. A similar recovery from hypertensive vascular damage would be difficult to demonstrate in man, but it might be presumed if it could be shown that renal function and albuminuria improved, or even if the survival period was prolonged as a result of hypotensive therapy.

In primary renal disease the influence of hypertension on the pathological process will vary according to the type of renal damage and in particular according to the relative contributions which the primary inflammatory lesion and secondary hypertensive changes make to the pathological process. In many cases of chronic Type I nephritis the symptomatology and course appear to be determined chiefly by the degree and stage of development of high blood pressure. Volhard (1931) classified these cases as the "vascular course of chronic nephritis" and Ellis (1942) emphasized the important role of hypertensive vascular changes in this form of Bright's disease. In the later stages the hypertension may change from the benign to the malignant type, and this transition appears to be much more common in renal than in essential hypertension. Wilson (1953) observed the malignant hypertension syndrome in 32 out of 65 cases of chronic Type I nephritis. It is in this form of nephritis that hypotensive drug therapy might be expected to delay the progressive course and to diminish the rate of renal deterioration. A very similar situation arises in

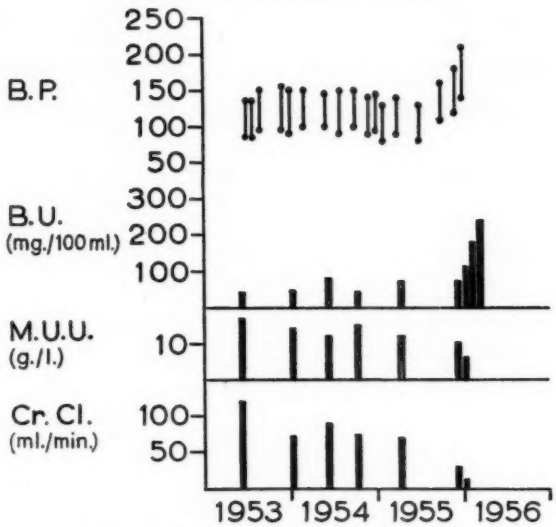
chronic pyelonephritis. Here again the primary inflammatory lesion in the kidneys has for the most part healed and secondary hypertension may be largely responsible for both symptoms and subsequent renal damage. When the lesion is confined to one kidney, dramatic relief is sometimes obtained, even in the malignant stage, by removal of the diseased kidney (Langley & Platt, 1947; Pickering & Heptinstall, 1953). When chronic pyelonephritis is bilateral, beneficial effects might therefore be expected if the blood pressure is lowered by hypotensive drugs, at any rate if renal function is relatively unimpaired.

On the other hand, it must be recognized that in many patients with chronic nephritis the kidney is severely and diffusely damaged by the initial inflammatory lesion. This is particularly true in the rapidly progressive course of Type I nephritis and in chronic Type II nephritis. In both these variants, hypertensive manifestations may be severe and the malignant termination is observed in over half the cases (Wilson, 1953). Nevertheless in such severely damaged kidneys, and particularly in chronic Type II nephritis, secondary hypertensive vascular and glomerular lesions are usually inconspicuous (Ellis, 1942). Whilst in these patients hypotensive therapy might be expected to produce symptomatic relief, e.g., from heart failure or hypertensive encephalopathy, it is unlikely that the prognosis would be improved—indeed it might be expected that renal clearance would be further impaired by lowering the blood pressure in diffusely damaged kidneys. There are other, rarer forms of diffuse renal disease, e.g., amyloid disease and polycystic kidney, in which hypotensive therapy might similarly be expected to produce little if any beneficial effect.

2. Indications for Treatment and Selection of Cases

From the foregoing considerations the indications for hypotensive therapy in renal disease may be regarded as palliative or prophylactic. Palliative therapy may be given to relieve headache, to combat heart failure—and by doing so renal function may be improved—or to counter an attack of hypertensive encephalopathy. Prophylactic treatment is directed to the prevention of hypertensive vascular damage, particularly in the kidney and retina, and is the more urgent when evidence of such vascular damage is already present. Thus the occurrence of retinal haemorrhages or the development of papilloedema are the most definite indications for lowering the blood pressure. All these considerations apply both to essential and renal hypertension. In renal hypertension, however, prophylactic treatment is justifiable even before retinal changes appear and the emphasis should be placed on a rising blood-pressure level rather than on the appearance of retinal changes. When patients with chronic renal disease are kept under regular observation, it is usual at some stage, often after many years, to observe a steep elevation of blood pressure; the pressure may rise from moderate levels to over 200 mm. Hg (systolic) over the period of a few months. This " hypertensive transformation " (Wilson, 1955) is usually accompanied by a marked deterioration in renal function, and in the absence of therapy malignant hypertension may supervene (fig. 1). It is at this stage, when the blood pressure rises steeply, that hypotensive drugs might be expected to produce their maximum prophylactic effect in postponing or retarding the development of renal failure and in preventing hypertensive retinopathy.

FIG. 1. "HYPERTENSIVE TRANSFORMATION" IN A CASE OF CHRONIC NEPHRITIS



B.P.: systolic blood pressure (mm. Hg)
B.U.: blood urea (mg./100 ml.)
M.U.U.: maximum urine urea concentration in 3-hr. concentration test (g./l.)
Cr. Cl.: creatinine clearance (ml./min.)

Note sudden deterioration in renal function closely associated with rapidly rising blood pressure.

3. Results of Treatment in Renal Hypertension

Reports in the literature on the results of hypotensive therapy in renal disease are few, and detailed renal function studies before and after treatment are even less common. Morrison (1953), reporting the results of parenteral hexamethonium therapy in hypertension, treated 12 cases of renal disease for 3–15 months. In seven, the blood urea was normal before treatment and no increase was observed during the period of follow-up. Five patients had raised blood-urea levels before treatment; three of these showed no further rise during treatment, whilst two showed a slow rise over many months. There was no evidence that hexamethonium had accelerated the progress of renal disease in these cases. In one member of the above series McMichael (personal communication) has observed survival for five and a half years after reversal of malignant hypertension, the blood urea remaining moderately elevated (90–105 mg./100 ml.). Harington & Rosenheim (1954) compared the effects of hexamethonium therapy on renal function in essential and renal hypertension. In 16 cases of essential hypertension (six benign and ten malignant), the blood urea fell slightly during the first year of hypotensive therapy; a similar fall took place in four out of five cases of renal hypertension. These observations have recently been extended (Harington, personal communication) to include 18 cases of renal hypertension, of which six were examples of chronic pyelonephritis and six of chronic glomerulonephritis. In 14 of this series, papilloedema was present and adequate control of the hypertension (standing blood pressure 150/100 mm. Hg three hours after a test injection) was ob-

tained in seven. In six of the seven the blood urea was normal or slightly raised (not more than 60 mg./100 ml.) before treatment, and five of these have survived for periods of 56, 46, 31, 21 and 19 months without further elevation of blood urea. From the above observations, we believe it is justifiable to infer that, in patients with renal hypertension which has entered the malignant phase, satisfactory control of the blood pressure may prevent deterioration in renal function, so long as the degree of nitrogen retention is minimal at the start of treatment. The same observers have also contrasted the survival of patients with essential and renal hypertension in the malignant phase. Table I shows this contrast over a three-

TABLE I. TREATMENT OF MALIGNANT HYPERTENSION WITH GANGLION-BLOCKING DRUGS (Harington, M., personal communication)

Number of survivors at 1, 2 and 3 years: in brackets, number of possible survivors

	1 year	2 years	3 years
Malignant essential hypertension	22 (32)	10 (27)	5 (17)
Malignant renal hypertension	6 (14)	3 (7)	2 (5)
All cases (essential and renal)			
Good renal function	20 (23)	6 (16)	5 (10)
Poor renal function	8 (23)	7 (18)	2 (12)

Note: Renal function "good" if initial blood urea less than 45 mg./100 ml.; "poor" if initial blood urea 45 mg./100 ml. or over.

year period of observation. The table also indicates the adverse effect of impairment of renal function (see also Rosenheim, 1954). In six patients in the series with renal hypertension the initial blood-urea level was 75 mg./100 ml. or more, and the longest survival in this group was seven months after the commencement of treatment. In most of these patients, however, satisfactory control of the blood pressure was not obtained.

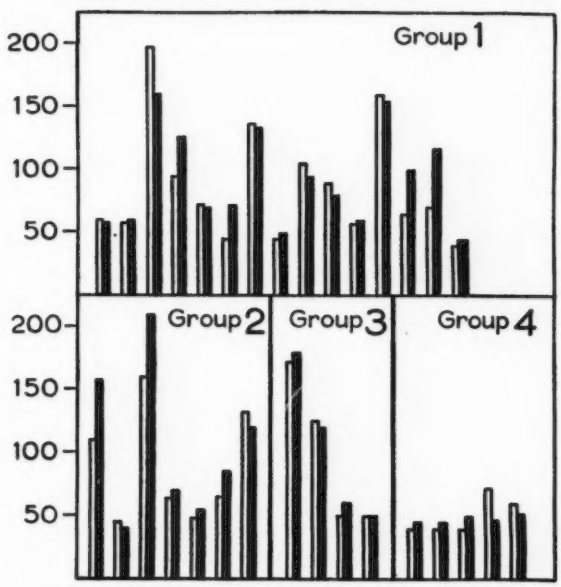
During the past three years we have studied the effect of hypotensive drugs on impaired renal function in patients attending the Nephritic Clinic of the London Hospital (Abrahams & Wilson, 1957). The objects of this investigation were (i) to determine in short-term studies whether the blood pressure could be lowered in chronic renal disease without further impairing renal function, (ii) to see whether, in the long term, renal deterioration could be retarded and malignant hypertension reversed or prevented, (iii) to study any differences in therapeutic response between the various types of chronic renal disease and malignant essential hypertension.

Observations have been made on 32 patients, the presumptive diagnosis being chronic Type I nephritis in 16, chronic Type II nephritis in seven, other forms of renal disease in four and malignant essential hypertension in five. Renal function was impaired in some degree in all cases initially. Papilloedema was observed in 12 patients and retinal haemorrhages or exudates, without papilloedema, in eight. At the start of the investigation Ansolysen (pentapyrrolidinium bitartrate) was given by injection; later, Ansolysen or Ecolid was given orally, usually in combination with Serpasil. At the start of treatment, short-term studies were made during a two- to four-week stay in hospital, and the blood pressure was recorded hourly for eight-hour periods to define the hypotensive response and to decide on the appropriate dosage. Renal function was investigated by periodic estimation of the blood urea, urine urea concentration, and creatinine clearance, before and after treatment.

a. Immediate Effects of Lowering the Blood Pressure

Figure 2 compares the blood-urea levels before treatment and after two to four weeks of effective reduction in blood pressure. The results are arranged according to diagnosis. In 15 trials in chronic Type I nephritis, renal function was unchanged in ten, worse in four and improved in one; the latter was probably due to relief of severe heart failure which was present before treatment. In seven cases of Type II nephritis, there was a significant rise in blood urea in three when the level of blood pressure was lowered, but in four there was no change. The patients whose condition deteriorated showed evidence of a continuing active nephritis, erythrocytes, leucocytes and granular casts being found regularly in the urine deposit. We cannot infer therefore that lowering the blood pressure aggravated renal failure in these patients, since rapid deterioration was taking place before the hypotensive drugs were administered. In the small group with

FIG. 2. EFFECT ON BLOOD UREA OF TWO TO FOUR WEEKS' REDUCTION IN BLOOD PRESSURE



Ordinates: blood urea (mg./100 ml.)

Each pair of columns represents one patient (with the exception that one patient in Group 1 is represented by two pairs of columns, two sets of observations having been made).

Open column: blood urea before treatment

Black column: blood urea after 2–4 weeks' reduction in blood pressure

Group 1: chronic Type I nephritis

Group 2: chronic Type II nephritis

Group 3: other forms of renal hypertension

Group 4: malignant essential hypertension

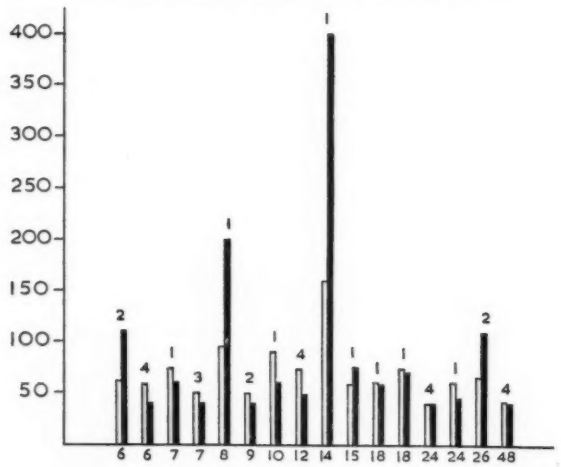
renal hypertension due to other causes, including pyelonephritis and amyloid disease, and in malignant essential hypertension, there was no evidence that acute reduction in blood pressure aggravated renal impairment.

b. Long-Term Results

Sixteen patients have been treated for periods of six months or longer. The initial and latest blood-urea values, with the time intervals, are indicated in fig. 3. Of eight cases with chronic Type I nephritis there was reduction in blood urea in three, no change in two and deterioration in three, the maximum period of observation being 24 months. This result appears to be better than might be expected in the absence of treatment, since papilloedema or haemorrhagic retinopathy was present in most instances. In all except one of the patients showing deterioration of renal function the initial creatinine clearance was below 35 ml./min. and the blood urea was above 60 mg./100 ml. In two cases of Type II nephritis, renal function deteriorated steadily during periods of 26 and six months' therapy respectively and in one case it has remained unchanged for nine months. Four patients with malignant essential hypertension have been treated for six months to four years without deterioration of renal function; either the blood urea or the creatinine clearance was initially within normal limits in all four.

In general it has been found that renal hypertension is satisfactorily controlled with smaller doses of ganglion-blocking drugs than are necessary in malignant essential hypertension. This may, of course, be due to impaired elimination of the drugs by damaged kidneys (Harington, 1953). Reversal of papilloedema appeared to be more rapid in primary renal disease than in malignant essential hypertension, disappearance of disk swelling being the rule in the former group within one month of the institution of hypotensive therapy.

FIG. 3. LONG-TERM EFFECTS ON BLOOD UREA OF REDUCTION IN BLOOD PRESSURE



Abscissae: months of treatment
Ordinates: blood urea (mg./100 ml.)

Each pair of columns represents one patient.
Open column: blood urea before treatment
Black column: blood urea at end of observation period
Figures over columns indicate diagnostic group (see fig. 2).

4. Conclusions

The effect on renal function of reducing the blood-pressure level in patients with hypertension due to primary renal disease has been inadequately studied. From the several small series here described certain general conclusions appear justified. It is apparent that renal hypertension can in most cases be adequately controlled by a combination of a ganglion-blocking drug and one of the rauwolfia alkaloids. When the malignant phase supervenes, the drugs may be more effective in reducing blood pressure in renal than in essential hypertension. Short-term studies indicate that acute reduction in blood pressure neither produces nor aggravates renal impairment when kidney function is normal or only slightly impaired. When renal function is severely impaired before treatment (e.g., blood urea over 60 mg./100 ml., creatinine clearance below 35 ml./min.), further deterioration is likely, but hypotensive therapy may still be justified for relief of symptoms. Moreover in the presence of heart failure, renal function may improve when the blood-pressure level is reduced. In the long term, hypotensive therapy is of limited value, but there is suggestive evidence that, when renal function is initially not more than slightly impaired, further deterioration may be retarded and retinal vascular damage may be prevented for a few years. It is likely that reduction of blood pressure will have the most favourable effect on prognosis in those forms of renal disease, such as chronic Type I nephritis and chronic pyelonephritis, where active inflammatory lesions are minimal and the hypertension is the main cause of symptoms and progressive renal damage. The least favourable results are to be expected in chronic Type II nephritis where the inflammatory lesion is active and progressive. Since the presence of renal failure has such an adverse effect, it is important that hypotensive therapy should be instituted as soon as the blood pressure is observed to rise from moderate to higher levels (e.g., 180 mm. Hg systolic), and before retinal haemorrhages and exudates or papilloedema have appeared. This prophylactic use of hypotensive drugs in the benign, asymptomatic phase of renal hypertension is entirely justified by the unfavourable course which is so commonly observed in the absence of treatment.

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THE NEPHROTIC SYNDROME

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1 Urinary protein losses and oedema
2 The size and nature of the clinical problem
3 The general and specific nature of the renal lesion
4 Prognosis and treatment
References

A diagnosis of " the nephrotic syndrome " implies a chain of events giving rise to oedema in a certain group of patients. Suspected on clinical grounds, it is confirmed by chemical tests which demonstrate severe proteinuria and a lowered serum albumin concentration. In addition, a number of other changes in serum proteins and lipids are characteristically found.

The most simple and rational explanation of the natural history of the syndrome is summarized diagrammatically in Table I. This implies that any proteinuria of sufficient duration and severity can produce the syndrome, which is now known to occur:

a. in systemic disease with secondary renal impairment (e.g. amyloidosis, disseminated lupus erythematosus, circulatory disorders, diabetes, etc.);
b. in primary renal disease
   i. associated with manifestations which are not the result of proteinuria (e.g. hypertension, diminished excretory function);
   ii. with proteinuria and consequent alterations in the serum as the only demonstrable abnormalities (the term " uncomplicated nephrotic syndrome " is sometimes used to describe patients in this category).

Later some demonstration will be given of the frequency of the various disease processes which may lead to the development of the nephrotic syndrome.

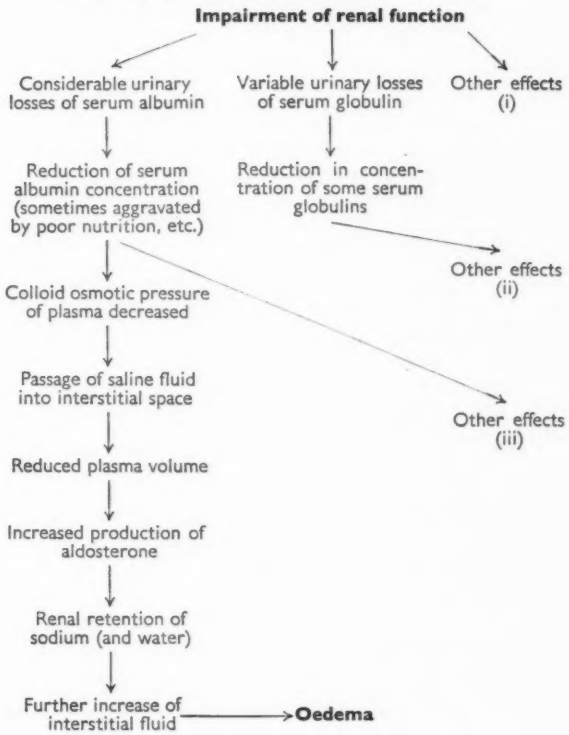
Almost all of the events pictured in the hypothetical sequence (Table I) have been called into question at various times. Thus, it has been suggested (cf. Bradley & Tyson, 1948):

i. the urine protein is not derived from the serum;
ii. the proteinuria is due to the presence of abnormal serum proteins and not to impaired renal function (Charlwood, 1952; Addis, 1945);
iii. the reduced serum albumin concentration is not related to the urinary loss;
iv. oedema is not related to the low serum albumin concentration and attendant reduction in plasma colloid osmotic pressure.

Though impaired protein-retaining power of the kidney is generally acknowledged to be the basis of the syndrome, there has also been debate as to whether the proteinuria is due to: (i) increased glomerular permeability; or (ii) diminished tubular reabsorption of protein passing through relatively normal glomeruli (cf. Rather, 1952).

Growth of interest in these questions during recent years has been stimulated by the advent of new techniques which are leading to definitive answers. These have come both from improved laboratory methods for separating and analysing proteins and from special forms of examination of patients which help to define more accurately the nature of the renal disease in each person affected. The hypotheses set out in Table I, several of which have been current for many years, seem to accord with these newer findings. Another major advance was the demonstration that short courses of cortisone or adrenocorticotrophic hormone (ACTH) lead to the dramatic

TABLE I. THEORETICAL SEQUENCE OF EVENTS LEADING TO OEDEMA AND OTHER FEATURES OF THE NEPHROTIC SYNDROME


Impairment of renal function
Considerable urinary losses of serum albumin
Variable urinary losses of serum globulin
Other effects (i)
Reduction of serum albumin concentration (sometimes aggravated by poor nutrition, etc.)
Reduction in concentration of some serum globulins
Other effects (ii)
Colloid osmotic pressure of plasma decreased
Passage of saline fluid into interstitial space
Other effects (iii)
Reduced plasma volume
Increased production of aldosterone
Renal retention of sodium (and water)
Further increase of interstitial fluid
Oedema

Note: Other effects may include:

i. Various manifestations of renal disease (e.g. hypertension, diminished creatinine clearance, abnormalities of renal tubular function, abnormal urinary deposits, etc.)
ii. Increased liability to infection; anaemia in some instances
iii. Wasting of body-protein reserves, e.g. muscle, liver protein

relief of oedema. With this form of treatment, relapse is common; it was also disappointing to find little or no reduction in mortality. With prolonged administration of cortisone, however, much better results have been reported in children (National Nephrosis Foundation, 1955), and in the following account we give our preliminary experiences with adults. These suggest that many patients can now be maintained symptom free, and their lives prolonged. Much more investigation is needed to define those diseases for which cortisone is indicated, or for which some other forms of treatment are useful.

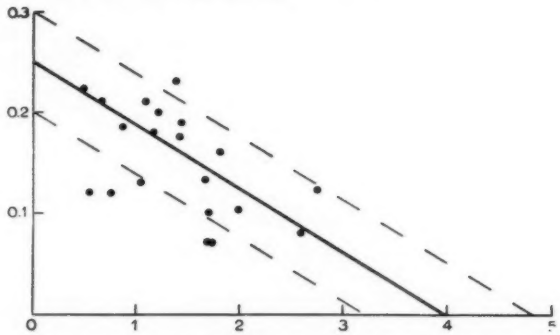
1. Urinary Protein Losses and Oedema

Electrophoresis on filter paper strips shows that urine proteins include five fractions, each moving at a rate which corresponds with that of a fraction found in serum. Occasionally a dense band of myeloma protein (" M-protein ") is found in urine; it is then also present in serum. In nephrotic urine, the albumin fraction accounts for 50–80% of the total proteins, even when the patient's serum proteins contain as little as 10% of albumin. On the other hand some fractions (e.g. α_2-globulin), composed mainly of large molecules, are usually poorly represented in the urine; staining electrophoretic strips for fat also demonstrates the absence from urine of lipoproteins; these are also very large molecules and are unusually abundant in nephrotic serum. The urine proteins, therefore, seem to be composed of serum constituents, though the larger molecules appear to escape with difficulty or not at all (Hardwicke, 1954).

Rowe (1956) has shown that ultrafiltration of normal or nephrotic serum through nitrocellulose membranes with moderate-sized " pores " leads to protein mixtures indistinguishable from nephrotic urine proteins. By electrophoresis on columns, he has separated albumin from three sources—nephrotic serum and urine, and normal serum—and shown that all have identical molecular weights. Immunological tests using precipitating antisera confirm this identity (Burtin & Grabar, 1954). By allowing urine and serum specimens to diffuse through gels and react with antisera, six or more individual urine proteins can be shown to be identical with serum constituents, though this test also shows that certain serum fractions are absent or poorly represented in urine (Gell, 1955). There now seems no reason to doubt that proteinuria represents a leakage of serum constituents, and that, apart from M-protein, these do not differ in kind as between normal and nephrotic serum.

Leakage of albumin for prolonged periods is invariably associated with a reduced serum albumin concentration, as shown in fig. 1 (cf. also Squire, 1952). Each point represents the relationship found in a patient with primary renal disease after a period of dietary treatment when proteinuria was maximal. With poor diets or with associated systemic disease (e.g. diabetes, disseminated lupus erythematosus),

FIG. 1. RELATIONSHIP BETWEEN REDUCED SERUM ALBUMIN CONCENTRATION AND DAILY ALBUMIN LOSSES IN URINE



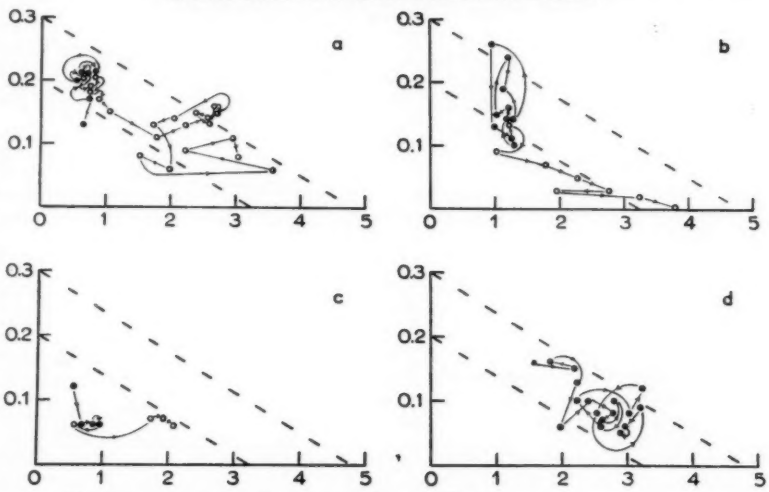
Abscissae: plasma albumin concentration (g./100 ml.)
Ordinates: albumin loss in urine per day (g./kg. body-weight)

The albumin was estimated in each case from paper electrophoresis and biuret estimation of total protein concentration.

One result is plotted from each of 20 patients with primary renal disease, the period of maximal sustained proteinuria being selected, which usually followed a period of high-protein feeding.

Continuous line corresponds with theoretical normal albumin production (Squire, 1955); interrupted lines are drawn to correspond with + 20% and − 20% of these values.

FIG. 2. RELATIONSHIP BETWEEN SERUM ALBUMIN CONCENTRATIONS AND URINARY ALBUMIN LOSSES



Abscissae: plasma albumin concentration (g./100 ml.)
Ordinates: albumin loss in urine per day (g./kg. body-weight)

Losses in 4 patients considered separately, showing successive determinations repeated at intervals over periods of many months.

● ● ● conservative treatment ○ ○ ○ cortisone treatment

Interrupted lines drawn as in fig. 1.

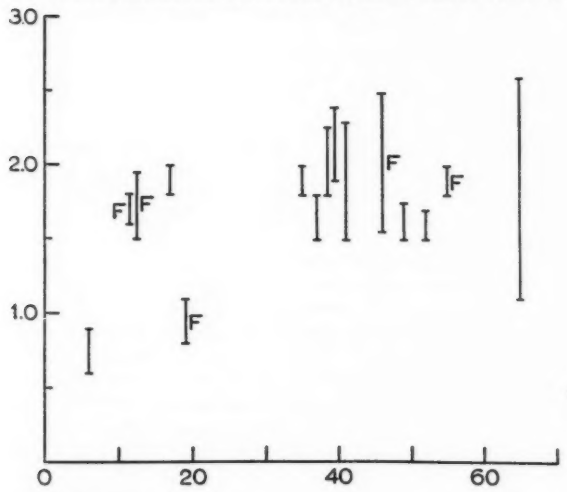
Figs. 2a, 2b, 2d (patients nos. 11, 6 and 7 respectively, Table III) show the usual sequence with diminution of albuminuria accompanied by increased serum albumin concentration. This holds true whether the albumin clearance is reduced by cortisone (2a, 2b) or falls with progressive renal failure (2d). Albumin production is apparently unaffected by cortisone treatment in 2a and 2b; in the exceptional example shown in 2c (patient no. 13, Table III) cortisone increased albumin production as well as reducing albumin clearance.

the serum albumin concentration may show even greater falls with similar protein losses; the findings under these special circumstances are best interpreted at present in terms of lowered albumin production. In patients with primary renal disease on a good diet, it appears that production is approximately normal, and balances the losses and utilization within the body, the latter being reduced in proportion to the serum albumin concentration (Squire, 1955). The relationship between urinary albumin losses and serum albumin concentration generally holds good in each patient even when the protein-retaining power of the kidney improves spontaneously or with cortisone treatment (fig. 2).

Oedema has long been known to depend principally on reduced serum albumin concentration. The critical level at which oedema is likely is shown in fig. 3; older results depended on less accurate methods for estimating albumin. Restriction of salt intake delays oedema formation, but in most patients does not prevent it when the serum albumin concentration falls below 1.6 g./100 ml. (i.e. 40% of the normal). Exceptionally, in young patients oedema may be absent with even more severe hypoalbuminaemia, no proved explanation being available. Comparison of serum albumin concentration with colloid osmotic pressure (fig. 4) in nephrotic subjects shows a close and roughly linear relationship. Oedema is also liable to occur, therefore, with a reduction in serum colloid osmotic pressure to below 40% of the normal value.

This short consideration of the serum protein changes in nephrotic patients omits reference to important work current in several countries which aims at determining the exact rates of turnover of each constituent protein. For the present, however, it is useful to regard the more important serum deficiencies as depending principally on protein leakage into the urine and as leading to the main symptom, oedema. Radical relief can be forecast from those forms of treatment which reduce proteinuria without reducing serum albumin concentration and without damaging the kidneys or other organs.

FIG. 3. RELATIONSHIP BETWEEN SERUM ALBUMIN CONCENTRATION AND OEDEMA IN 15 NEPHROTIC PATIENTS WITH PRIMARY RENAL DISEASE



Abscissae: age (years)
Ordinates: plasma albumin concentration (g./100 ml.)

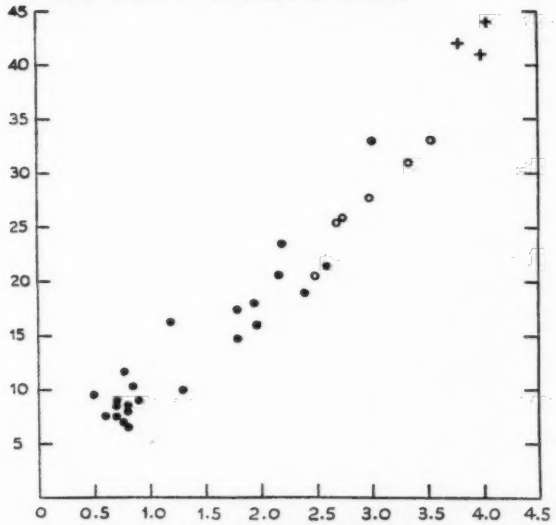
Lower limits of lines: highest serum albumin when clinical oedema was present

Upper limits: lowest serum albumin when patient was oedema-free

F: females

The limiting level of serum albumin below which oedema occurs is apparently about 1.6 g./100 ml., except occasionally in young people. This value may, of course, be higher when the nephrotic state is complicated by venous congestion (e.g. cardiac failure), or by abnormal capillary permeability to protein.

FIG. 4. RELATIONSHIP BETWEEN SERUM ALBUMIN CONCENTRATION AND COLLOID OSMOTIC PRESSURE OF THE WHOLE SERUM



Abscissae: serum albumin concentration (g./100 ml.)
Ordinates: serum colloid osmotic pressure (cm. water)

● ● ● nephrotic sera
○ ○ ○ nephrotic sera following albumin infusions } 7 patients, 30 determinations

+ + + 3 representative normal subjects

In the nephrotic syndrome, by chance, the relationship between serum albumin concentration and colloid osmotic pressure of the whole serum is direct, presumably as the result of higher globulin concentrations in patients with the greatest reduction in albumin concentration. Since serum albumin of 2.0 g./100 ml. corresponds to colloid osmotic pressure of just under 20 cm., and albumin of 1.0 g./100 ml. to colloid osmotic pressure of just under 10 cm. water, the findings in fig. 3 can be translated into terms of colloid osmotic pressure. Oedema is likely if the serum colloid osmotic pressure falls below 15 cm. water, except occasionally in young people.

2. The Size and Nature of the Clinical Problem

Deaths from nephritis have been classified since 1950 by the Registrar General as:

i. acute nephritis;
ii. nephritis with oedema, including nephrosis;
iii. chronic nephritis.

Of these, category (ii) probably corresponds with the nephrotic syndrome, though some examples of deaths from this cause may well be classified under a separate aetiological heading

TABLE II. DEATHS AND MORTALITY FROM NEPHRITIS WITH OEDEMA, INCLUDING NEPHROSIS, IN ENGLAND AND WALES, 1950–54

Calculated from annual reports of the Registrar General

Age-group (years)	Number of deaths										Deaths per million living									
	Year: 1950		1951		1952		1953		1954		1950		1951		1952		1953		1954	
	Sex: ♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0–19	43	29	34	27	30	15	22	15	25	19	7.1	4.8	5.4	4.4	4.7	2.4	3.4	2.4	3.7	3.0
20–39	43	43	31	26	42	29	36	23	28	19	7.0	6.7	5.0	4.1	6.9	4.6	5.9	3.7	4.6	3.1
40–59	69	64	57	45	55	52	68	35	61	45	12.4	10.5	10.0	7.3	9.6	8.4	11.8	5.6	10.4	7.1
60 and over	81	95	86	94	72	52	77	63	65	64	27.6	23.8	29.5	23.2	24.5	12.6	26.0	15.0	21.8	15.0
Total	236	231	208	192	199	148	203	136	179	147	11.33*	10.36*	9.90*	8.59*	9.41*	6.52*	9.56*	5.96*	8.33	6.38
Total, both sexes	467		400		347		339		326		10.83†		9.22†		7.92†		7.69†		7.32	

* These total mortality rates have been standardized for age distribution of the 1954 population.
† These combined totals have been standardized as for the total mortality rates and for the sex ratio in the 1954 population.

(e.g. myelomatosis). From 1950 to 1954, an average of 376 persons died each year from " nephritis with oedema, including nephrosis ", a considerably smaller number than from " chronic nephritis " though rather more than from " acute nephritis ". On an average about 12 men died for every 10 women under each of the nephritis headings (i) and (ii). In Table II, the detailed figures are displayed together with the mortality rates, which are much greater in the older age-groups. The mortality rate has been falling steadily, and that for 1954, after allowance for small changes in the composition of the population, is shown to be only two-thirds of the 1950 rate. This change is thought to reflect a real fall in mortality, since diagnostic criteria are not likely to have changed greatly and the other categories of nephritis have shown similar falls. The figures do not of course show whether the incidence of the diseases concerned is falling or whether improved treatment has led to the smaller numbers of deaths. But, with methods now available, it is considered that a much more rapid fall in death-rate from the nephrotic syndrome should be attainable during the next five years.

For comparison with the national figures a list is given in Table III of all 44 patients with nephrotic oedema referred to this Department for detailed study during the period June 1952 to June 1956. All but two of these patients were resident in greater Birmingham (population about 1,000,000) and, during this 5-year period, 20 patients have died. If one assumes that the mortality in Birmingham resembles that in the remainder of England and Wales, this series may represent between one-quarter and one-tenth of all such cases occurring in the area. Apart from the fact that all the patients but one were 12 years or more in age, there is no known selection, other than by symptom (oedema) and chemical criteria (continued proteinuria; serum albumin at some time less than 2 g./100 ml.).

Details are given for each patient, since these may allow comparison with other series. An attempt has been made to classify these patients in terms of diagnosis; though the details of such a classification will remain in dispute until more exact aetiological factors can be defined, the syndrome can certainly be regarded as arising in a variety of ways. A broad attempt has been made to divide the patients into those who primarily have renal disorders, and those who show the nephrotic syndrome complicating more generalized diseases; this leads to an almost equal division of numbers. Of the primary renal disorders, all but one of the patients were under 60 years of age, and males were heavily preponderant; of the remainder, nearly half were 60 or over, and the sexes were equally represented. The case mortality was greater in the older age-group (60 upwards) and lower for the primary renal disorders (even allowing for age differences). The case mortality is also apparently greater in men than women. Factors thought to be of prognostic significance (creatinine clearance, packed cell volume, blood pressure) have also been listed and will be discussed below.

Clearly, the nephrotic syndrome consists of an assembly of diverse conditions, united only in the common symptom, oedema, and by the mechanism of its formation. For symptomatic treatment, several measures (diet, salt restriction, blood-pressure control) may be useful in all of these patients; for more specific and radical relief, precise diagnosis is required.

3. The General and Specific Nature of the Renal Lesion

Protein is present only in traces in normal urine. Experimental work in animals has recently established that the renal tubules normally reabsorb some protein which is presumed to have passed the glomerular filter; in rats, this may be equivalent in one day to one-third of the total circulating serum albumin (Sellers, Griggs, Marmorston & Goodman, 1954). Proteinuria, therefore, might theoretically result from failure of tubular reabsorption, from increased permeability of the glomerulus, or from a combination of both of these factors.

In favour of increased glomerular permeability is the demonstration of protein in the capsular space when biopsy material is examined histologically. In acute nephritis and in some examples of disseminated lupus erythematosus, the similarity of the clearances of high molecular weight proteins (α_2-globulin, γ-globulin) to those of albumin indicates the loss of selective permeability and, almost certainly, gross damage to the glomerular membrane. A high protein content in the oedema fluid in these conditions would suggest a generalized capillary damage; evidence for this is, however, inconclusive (cf. Bodansky & Bodansky, 1952). In the nephrotic syndrome, the glomerular membrane remains highly selective, and the mean molecular weight of the proteins in the urine is even lower than that of albumin (Rowe, 1956); the glomerular membrane may appear simply thickened or even normal, whereas the tubules, as is well known, are often loaded with lipid.

In an attempt to distinguish between glomerular and tubular malfunction, patients have been infused with serum albumin so as to raise the serum albumin concentration for a time. Results have been published by two groups of workers (Chinard, Lauson, Eder, Greif & Hiller, 1954; Hardwicke & Squire, 1955). The analysis of the results is indirect, but is essentially analogous to the determination of tubular mass in relation to a substance known to be reabsorbed (e.g. glucose). In children with the nephrotic syndrome, no definite reabsorption was demonstrated (Chinard *et al.* 1954). In young adults with subacute nephritis, considerable reabsorption of all protein fractions was inferred, the amounts differing from patient to patient (Hardwicke & Squire, 1955). Both series demonstrated glomerular permeabilities to albumin which were greater than the maximum thought to be normal. It seems probable, therefore, that some degree both of abnormal glomerular permeability and of diminished reabsorption may be concerned with the proteinuria of the nephrotic syndrome. Further tests of this kind, using even more specific methods for estimating the concentrations of proteins in urine and serum, may show more precisely how these clearances depend on these two aspects—glomerular and tubular—of renal function.

Specific diagnosis of the diseases which give rise to the nephrotic syndrome is important, but often difficult. Clinical examination and established tests will lead to the recognition of some of the less common causes such as diabetes, constrictive pericarditis, and some examples of amyloidosis and disseminated lupus erythematosus. Renal vein thrombosis may be suspected when signs of femoral or caval thrombosis are present. Knowledge of various histological abnormalities in the kidney has been obtained until recently mainly from autopsy material, which is often complicated by terminal effects and by changes (caused for example by hypertension) which are common to many different conditions. Needle biopsy of the kidney has now been shown to be a safe and simple procedure (Kark, Muehrcke, Pirani & Pollak, 1955), and may where necessary be repeated to determine progress. There is much to be learned about the interpretation of these specimens; already, valuable diagnostic information is to be obtained in cases suspected of amyloid disease, lupus erythematosus, diabetes and chronic pyelonephritis. Gross structural changes such as renal enlargement, congenital or vascular abnormalities and hydronephrosis, can be discovered, sometimes unexpectedly, by radiological examination; this is increasingly useful with technical improvements in intravenous pyelography and renal arteriography. Recent advances enabling the renal veins to be examined (e.g. for thrombosis) are described elsewhere in this Bulletin.[1]

Quantitative examination of blood and urine (4-hour or 24-hour specimens) also assists in diagnosis. Serum complement is lowered in glomerulonephritis, as in some forms of experimental nephritis (Lange, Slobody & Strang, 1955). This finding has been interpreted as depending on continued antigen-antibody reactions within the body; on occasion it is valuable as an index of progress, especially to determine the adequacy of dosage with cortisone or related drugs for suppressing these reactions.

Some degree of blood eosinophilia is found in about one-third of nephrotic children (McCall, Ross, Wolman, Burns, Harpur & Goldbloom, 1952; cf. also Luetscher & Deming, 1950), though its significance is unknown. In case number 14 (Table III), more than 1,000 eosinophils/mm.^{3} were found, and skin tests showed strong reactions to pollens (trees and grasses). Recurrent summer relapses of nephrotic oedema suggested that these findings were more than coincidental.

Enumeration of formed elements in the urinary deposit (Addis, 1948) is sometimes useful in suggesting the presence of pyelonephritis, in which excessive numbers of pus cells are often, though not invariably, seen. Red cell losses of over 100 million daily are seen in renal vein thrombosis, acute nephritis and other conditions; repeated counts of 20–100 million, with occasional cellular and some granular casts, help to distinguish subacute nephritis from the uncomplicated nephrotic syndrome with lower counts. Increased glomerular permeability to the larger serum protein fractions (α_2-globulin, γ-globulin) is best expressed quantitatively in terms of globulin clearances relative to albumin clearances; high relative globulin clearances indicate severe glomerulitis (acute nephritis, disseminated lupus erythematosus), while intermediate values (e.g. γ-globulin clearances *ca* 30% or more of albumin clearance) are common in patients with subacute nephritis (Hardwicke & Squire, 1955). It must be admitted that there is no sharp dividing line between the different forms of primary renal disease on any single test or examination. Serial studies over prolonged periods are desirable in all patients with continued proteinuria.

4. Prognosis and Treatment

The prognosis in the nephrotic syndrome has been regarded as gloomy, epecially since 1942 when Ellis reported that, of a series of 145 patients with " Type II nephritis ", recovery was observed in only five. Similar findings were given by Davson & Platt (1949) though, in their series, the outlook in the absence of hypertension seemed somewhat better. Other reports have suggested lower but still formidable mortality rates; only in the nephrosis of children has complete recovery of about one-third of the cases been reported (cf. Barnett,

[1] See Steiner, p. 64 of this number of the Bulletin.—Ed.

TABLE III. VARIETIES OF THE NE

Details of all 44 patients studied 19

Criteria for inclusion: (i) Oedema with serum albumin below 2 g.

Variety	Case number	Sex	Age at onset (years)	Duration of nephrotic syndrome before observation started (months)	Period of observation (months)	Outcome	Remarks	Interval between first observation and start of continuous oral cortisone and penicillin (months)	Other si
Primary renal disorders: Acute nephritis	1	♂	36	0	5	Death	Autopsy	—	
Subacute nephritis	2	♂	37	0	7	Death	Autopsy	—	
	3	♂	31	3	12	Death	Autopsy: disseminated sclerosis	—	
	4	♂	50	6	4	Death	Autopsy	—	
	5	♂	45	2	7	Oedema +, not working	—	—	
	6	♂	49	1	25	Fit for work	—	12	Reserpine
	7	♀	12	6	32	Working sporadically	Biopsy (December 1955): chronic glomerulonephritis	24	Reserpine
	8	♂	41	0	16	Well, working	Pernicious anaemia	3	Reserpine
	9	♂	20	2	1	Death	Autopsy: chronic rheumatic heart disease with congestive cardiac failure	—	
Uncomplicated nephrotic syndrome	10	♂	41	0	21	Well, working	—	19	
	11	♂	17	53	17	Well, working	Skin hypersensitive to pollens	2	Reserpine
	12	♂	52	1	11	Well, working	—	1	
	13	♂	6	11	8	Well	—	4 (then intermittently for 4 extra months)	
	14	♂	39	76	6	Well, working	Eosinophilia, skin hypersensitive to pollens and dust	0	
	15	♀	12	18	29	Oedema-free	—	—	
	16	♀	19	2	29	Death	Autopsy	—	
	17	♀	46	4	15	Well, working	—	4	
	18	♀	55	0	5	Oedema present	—	1	
	19	♀	25	10	9	Oedema present	Biopsy: normal kidney	1	
Chronic nephritis	20	♂	47	42	8	Death	Congestive cardiac failure	—	
	21	♂	65	2	3	Death	—	—	
	22	♂	18	23	3	Death	Autopsy. Nephrectomy for fibrotic (? congenital) left kidney	—	
Congenital renal abnormality	23	♂	39	2	28	Oedema-free, working	Congenital rotation of kidneys; left hydronephrosis	25	Tetracycline reserpine
Nephrotic syndrome complicating: Diabetes	24	♀	53	2	29	Well, working	Diabetes, congestive cardiac failure	—	
	25	♂	38	4	16	Death	Autopsy: diabetic glomerulosclerosis. Hypophysectomy	—	
Disseminated lupus erythematosus	26	♀	31	2	1	Death	Autopsy: uraemia, endocarditis, disseminated lupus erythematosus involving kidney	—	
	27	♂	30	0	2	Death	Discoid lupus erythematosus, 4 years. Autopsy: disseminated lupus erythematosus involving kidney	—	
	28	♂	28	2	2	Improved	Acute disseminated lupus erythematosus	0	Various anti
Secondary amyloidosis	29	♂	22	0	1	Oedema-free	Still's disease treated with gold. Ruptured spleen removed February 1956: diffuse amyloid	—	
Myelomatosis with primary amyloidosis	30	♂	61	3	3	Death	Biopsy of gums, bone: amyloidosis, myelomatosis. M-protein in urine and serum	—	
Myelomatosis	31	♂	68	8	15	Deteriorating, still some oedema	M-protein in urine and serum. No other evidence of myelomatosis	7	Reserpine
	32	♂	52	24	1	Death June 1955	M-protein in urine and serum	—	
	33	♀	75	5	4	Oedema present	M-protein in urine and serum. Sternal puncture: myelomatosis. (Pernicious anaemia—1953)	1	
Carcinoma of prostate	34	♂	69	3	6	Death	Carcinoma of prostate, congestive cardiac failure	—	
Congestive cardiac failure	35	♀	58	6	26	Well	Constrictive pericarditis	—	Pericardial st
	36	♂	63	0	6	Oedema-free	Angina 2 years. Coronary infarction and congestive cardiac failure, October 1955	—	
	37	♂	62	6	1	Death	Autopsy: coronary thrombosis	—	
Renal vein thrombosis	38	♀	55	1	9	Death	Congestive cardiac failure	—	
	39	♂	73	2	3	Death	Autopsy: renal vein thrombosis	—	
	40	♂	28	5	3	Oedema-free	Traumatic thrombosis of inferior vena cava. Biopsy: Type II nephritis	2	Anticoagulan
	41	♀	58	2	2	Still oedematous	Femoral thrombosis. ? Renal vein thrombosis	—	
	42	♀	61	2	3	Death	Autopsy: congestive cardiac failure, renal vein thrombosis	—	
Chronic pyelonephritis	43	♀	48	76	2	Death	Autopsy	—	Short course tetracycline,
	44	♀	45	2	16	Well, working	Biopsy: normal kidney	2	

* At one time intermediate creatinine clearance was 16 ml./min./70 kg., packed cell volume 29, and blood pressure readings 170/120 mm. Hg.

THE NEPHROTIC SYNDROME

udied 1952 to June 1956

elow 2 g./100 ml.; (ii) continued proteinuria

Other significant treatment	Creatinine clearance (ml./min./70 kg.)		Packed cell volume		Blood pressure (mm. Hg)		Publications giving further details of case history
	First	Last	First	Last	First	Last	
	15	—	Anaemia present	—	170/100	—	
	44	2	40	20	190/100	220/120	Case 4 in Hardwicke & Squire (1955)
	49	12	35	20	160/90	235/135	Case 5 in Hardwicke & Squire (1955)
	18	4	31	20	185/105	205/100	
	18	47	36	37	160/100	160/100	
Reserpine	15	30	42•	44	140/85•	110/80	
Reserpine	58	19	26	27	130/90†	130/85	Case 6 in Hardwicke & Squire (1955); cf. also Blainey (1954)
Reserpine	52	75	30	45	160/110	160/95	
	—	51	—	53	—	190/30	
Reserpine	95	90	48	47	145/85	130/80	
	64	89	37	45	125/90	160/115	
	50	88	45	46	170/110	140/80	
	101	140	35	38	110/80	110/80	
	70	80	40	43	110/70	140/100	
	91	(Blood urea, 46 mg./100 ml.)	40	33	125/90	170/115	Case 17 in Hardwicke & Squire (1955)
	147	32	46	20	125/80	170/112	Cf. Blainey (1954)
	70	77	35	40	190/95	140/80	
	110	29	38	38	—	150/90	
	124	110	40	46	135/85	120/80	
	—	11	—	51	240/140	185/112	
	—	6	—	35	210/110	165/100	
	11	9	27	15	170/108	—	
Tetracycline, sulphamerazine, reserpine	60	49	43	31	145/95‡	150/80	
	47	—	28	—	170/80	140/90	
	—	25	—	40	—	260/140	
	59	(Blood urea, 432 mg./100 ml.)	—	21	—	144/120	
	46	27	32	22	156/100	120/86	
Various antibiotics	103	111	37	33	160/80	170/90	
	21	—	32	—	100/70	—	
	75	74	39	40	120/70	90/60	
Reserpine	65	11	48	42	170/90	150/90	
	100	—	31	—	150/100	—	
	58	73	36	36	140/90	145/85	
	63	31	41	34	220/130	180/120	
Pericardial stripping, December 1952	16	87	50	40	90/70	150/80	Case 3 in Blainey, Hardwicke & Whitfield (1954)
	18	—	51	—	180/70	—	
	—	24	—	33	—	195/115	
	93	18	36	42	120/60	160/100	
	—	6	—	31	—	200/120	Case 1 in Blainey et *al.* (1954); cf. also Blainey (1954)
Anticoagulants since March 1956	119	100	44	43	140/80	140/80	
	21	81	43	—	150/90	—	Case 2 in Blainey et *al.* (1954)
	25	—	52	—	170/100	—	
Short course chloramphenicol tetracycline, December 1955	—	3	—	35	—	210/110	
	114	46	40	40	120/80	120/80	

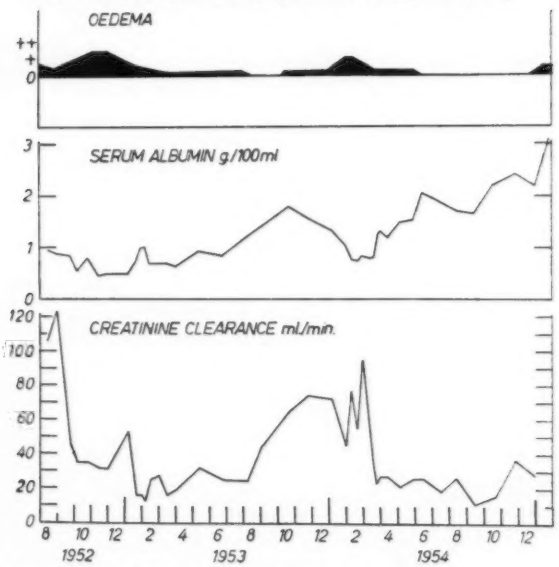
† Intermediate blood pressure—155/110 mm. Hg

‡ Intermediate blood pressure—180/100 mm. Hg

Forman & Lauson, 1952), though until recently equal numbers of the remainder had died within three years or showed continual signs of active renal disease. The association of hypertension, haematuria (either macroscopic or microscopic) and a rising non-protein nitrogen concentration in the blood have rightly been taken as evidence of progressive renal disease. Salt restriction, high-protein diets, and infusion of albumin and other colloids have combined to reduce somewhat the period of invalidism from oedema: some lives have been saved by the use of chemotherapy and antibiotics, especially of penicillin, but none of these measures appears to have influenced markedly the continued renal damage and ultimate outcome.

Prognosis in the individual patient is difficult, and prolonged observation may be needed to assess the condition. Whereas the proteinuria and serum albumin concentrations determine the presence of oedema, renal function as a whole largely determines the prognosis. Serial estimations of creatinine clearance have formed the most valuable index of renal function in our series; fig. 5 shows the values found, in comparison with the fluctuations in oedema and serum albumin concentration, in a young woman between the ages of 19 and 21. She was well in June 1952, but her condition was thereafter typical of the nephrotic syndrome, and the treatment was conservative throughout. The initial fall in clearance was followed by considerable recovery in the summer of 1953, while the serum albumin also improved. A minor intercurrent infection in November 1953 was at first followed by marked oscillations of creatinine clearance, and then by a severe and apparently irreversible fall. Though the serum albumin showed further improvement, the blood pressure rose to

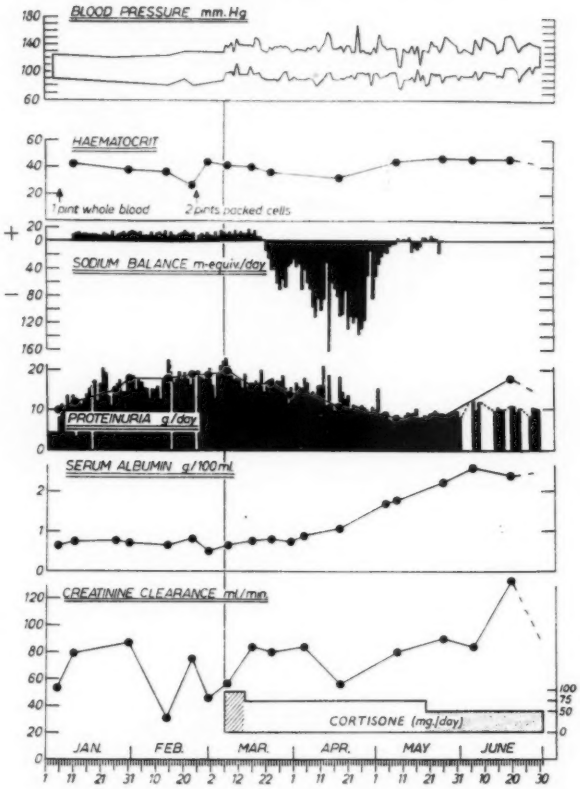
FIG. 5. COURSE OF THE NEPHROTIC SYNDROME IN A YOUNG WOMAN (patient no. 16, Table III)



Conservative treatment given throughout.

Insidious onset of illness in July 1952. First and second episodes of oedema were accompanied by usual degree of hypoalbuminaemia. (Terminal attack in January 1955 was complicated by heart failure.) Precipitous fall in creatinine clearance within 3 months of onset was followed by substantial recovery during 1953. Mild intercurrent infection in November 1953 was followed, after some oscillation in clearance level, by renewed fall to critically low level. The creatinine clearance did not improve during 1954; with developing hypertension in this year, prognosis was evidently grave. Further infection in January 1955 led to death within 2 weeks.

FIG. 6. COURSE OF THE NEPHROTIC SYNDROME IN A YOUNG MAN (patient no. 11, Table III)



Illness was of 4 years' standing with recurrent massive oedema. Treatment was conservative in January and February, and high-protein, high-calorie diet was given throughout. During period of conservative treatment, anaemia recurred after transfusion, oedema accumulated, proteinuria increased with severe hypoalbuminaemia persisting, creatinine clearance was oscillatory. Treatment with cortisone led to progressive though gradual fall in proteinuria, with diuresis (as indicated by reversal of sodium balance) starting after 16 days. Fall in haematocrit during this period was shown to be accompanied by greater plasma volume (T-1824 determinations); the patient then recovered from anaemia without further transfusion or specific treatment. Rise in serum albumin concentration was evident when diuresis was half completed. Creatinine clearance rose (interrupted temporarily by minor short relapse in October). Blood pressure rose with cortisone treatment, but from October was controlled by reserpine, 0.5 mg. daily. Strongly positive nitrogen balances were recorded throughout period covered by chart.

Note: In proteinuria chart, blocks represent determinations on 24-hour specimens; circles are calculated from 4-hour specimens obtained for creatinine clearances, so that correspondence or otherwise checks accuracy; e.g., last readings in June suggest over-collection of 4-hour specimen, and somewhat exaggerated value for creatinine clearance.

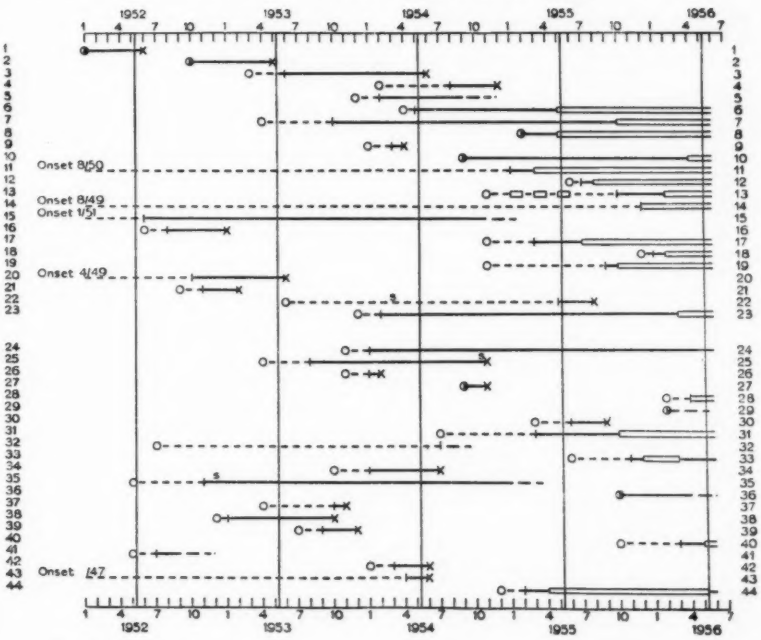
170/112 mm. Hg, and the patient died after 30 months of illness, following another infection in January 1955. In the early stages of such an illness, damaged renal function can, apparently, be followed by repair; but a continued depression of clearance to one-quarter of the normal level and, especially, a progressive fall in clearance, are ominous signs. Success in treatment should be judged not only by symptomatic relief (loss of oedema associated with return of serum proteins and lipids towards normal) but also in terms of survival (with the blood pressure controlled, the fall in creatinine clearance halted, and other signs of activity, such as anaemia and depressed serum complement, eliminated). These criteria are not fully attained by any known form of treatment in all types of disease leading to the nephrotic syndrome, but considerable progress is now apparent.

Short courses of cortisone or ACTH have been shown to produce diuresis and some reduction in proteinuria in the majority of patients, but relapses are frequent. It has previously been reported that continued dosage (on all or several days of every week) may lead to a considerably improved prognosis in children (National Nephrosis Foundation, 1955). In our series of adult patients, continuous moderate dosage with oral cortisone (usually 1–2 mg./kg. body-weight) has been used in 17 of 22 nephrotic patients with various diseases under observation during 1955–56 (Table III). This has led to a gradual progressive reduction in proteinuria in many cases, followed by diuresis and a rise in serum albumin concentration. The time taken to achieve these results has varied considerably; diuresis, for example, has occurred between seven days and seven months after starting treatment. The fall in albumin clearance observed in all of the successfully treated patients has not been accompanied by any fall in creatinine clearance, and the results can be interpreted as a gradual increase in the protein-retaining power of the kidney. Microscopic haematuria has diminished in several patients, and evidence of improved tubular function was found in one instance—a generalized aminoaciduria found repeatedly over a period of nine months disappeared within four months of starting treatment and has not returned during nine months of continued treatment. Figure 6 shows a typical response in a young man of 21 with a four-year history of proteinuria with recurrent attacks of massive oedema. Apart from a two-week relapse in October 1955, he has remained well and continuously at work since September 1955.

To avoid complicating infections, all patients have taken 500,000 units of penicillin orally twice daily, and a high-protein, high-calorie, high-calcium diet has been maintained. In those cases with diastolic blood pressures of 100 mm. Hg on more than two occasions, rauwolfia alkaloid (reserpine, 0.5—1.5 mg./day) has been given, with apparently beneficial effect. Of 13 patients observed long enough on continuous cortisone for satisfactory assessment, seven have shown considerable reduction in proteinuria and complete symptomatic relief; all of these were affected by primary renal disease. Six patients with the following diagnoses have failed to respond—advanced subacute nephritis, uncomplicated nephrotic syndrome (two cases), myelomatosis (two cases), chronic pyelonephritis (in this case, a mild anaemia responded to cortisone; the proteinuria subsequently disappeared after treatment with tetracycline). The effective dosage has varied from patient to patient, being determined individually by initially low dosage (50 mg. or 75 mg. in adults), with subsequent increase when necessary. The highest dosage relative to body-weight was required in the youngest patient of the series, aged six; 1.5 mg./kg. was ineffective, and success was achieved with 3.0 mg./kg.

No complications have been observed with this form of combined treatment other than nasal congestion and mental depression from reserpine (one case). A number of patients

FIG. 7. OUTCOME AND SPECIFIC TREATMENT IN 44 UNSELECTED PATIENTS WITH NEPHROTIC SYNDROME



o: onset
x: death
s: surgical intervention
– – – –: period of illness not under observation (if death had occurred during this period, patient would not have been recorded in the series)
|————: entry into series and period of observation with conservative treatment
|═══: observation, with cortisone + oral penicillin treatment

Patients are numbered as in Table III, where further details are available. Hence nos. 1–23 are of primary renal disease. The duration of survival is very variable, even within groupings of specific diseases. This chart indicates improved outlook during 1955–56, when continuous treatment with cortisone was used (for discussion, see text). Further follow-up continues (i) to determine ultimate outcome, and (ii) to define the efficacy of cortisone in different types of nephrotic syndrome.

have gained considerably in weight, owing in some degree to recovery in musculature but mainly to accumulation of generalized fat (Squire, 1956). Excess fat has usually been lost on the resumption of full activity, and some reduction in fat intake has led to slimming in others. In view of the very large losses of body-protein which occur, the importance of long-continued high-protein, high-calorie diets during the oedematous phase of the disease has been emphasized (Blainey, 1954; Squire, 1956). This type of diet, with the addition of high calorie intake, may be important also in avoiding adverse effects even from moderate cortisone dosage.

In one of our patients (case number 8, Table III), ACTH in dosage of 100 mg./day (1.3 mg./kg./day) led to a very considerable breakdown of body-protein as deduced from nitrogen balance studies, and, to judge from published accounts of blood urea or non-protein nitrogen level, this may be a frequent result of current dosage with this hormone (Metcoff, Rance, Kelsey, Nakasone & Janeway, 1952). No diminution in positive nitrogen balances has been found with cortisone in the dosage used in this study.

Prolonged observation is needed to assess the long-term outlook with cortisone therapy. At present, a prolongation in the term of life, as well as a restoration of fitness, seems consistent with the findings. In all patients successfully treated (numbers 6, 8, 11, 12, 13, 14 and 17—Table III), the creatinine clearance has shown a gradual tendency to rise. The blood pressure has been satisfactorily controlled, though in case number 11 it has risen somewhat since reserpine was temporarily discontinued. To assess mortality, the results in all 44 patients have been displayed in fig. 7. In crude terms the deaths may be related to the numbers of patients under observation in each year as follows: pre-cortisone 1952–53, 4 of 8; 1953–54, 6 of 16; 1954–55, 7 of 20; cortisone 1955–56, 2 of 22 (or 3 of 22 including case number 32 not under treatment at the time of death). The fallacies inherent in this form of retrospective analysis have been well exposed by Bradford Hill (1951). Furthermore, even if these results can be considered as evidence for a reduced mortality, the simultaneous introduction of three drugs (cortisone, penicillin and, in some cases, reserpine) makes interpretation especially complicated. Nevertheless, it seems that a therapeutic trial, controlled by patients from whom cortisone is withheld, is now difficult to justify. In view of encouraging short-term results with newer compounds (e.g. prednisolone (Arneil, 1956)), a careful comparison between alternative drugs might well be undertaken.

In spite of the advances of the last few years, the differential diagnosis of the nephrotic syndrome remains difficult. This being so, it is impossible to give a confident prognosis, or to claim that any treatment is truly curative. For the moment all cases on treatment have to be kept under hospital surveillance, since the assessment of progress depends so much on laboratory tests; it is also sound practice to observe regularly all cases of heavy proteinuria, if possible until a diagnosis is finally established.

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THE PATHOGENESIS OF URINARY CALCULUS FORMATION

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1 Associated conditions
- *a* Increased excretion of calcium in the urine
- *b* Urinary infection
- *c* Urinary stasis
- *d* Vitamin A deficiency
- *e* Randall's plaques
- *f* Microliths
- *g* Hydrogen-ion concentration of urine

2 Current theories of calculus formation
- *a* Deficiency of some substance or substances in urine, which normally prevent precipitation of calcium
- *b* The presence of some substance in the urine, which enhances the precipitation of calcium salts
- *c* A disturbance in the mechanism which normally rids the kidney of precipitated calcium

3 Summary
References

The majority of calculi are largely composed of calcium salts, and we will consider only this type.

Although we are ignorant of the basic disturbance in calculus formation we do know that stones are particularly likely to occur in association with certain conditions, such as urinary tract infection, hypercalciuria, etc. These associated conditions in themselves cannot account for calculus formation, as they occur frequently without calculi, but they must be considered in any hypothesis. They will be discussed first and followed by a consideration of the current views on the pathogenesis of stone formation.

1. Associated Conditions

a. Increased Excretion of Calcium in the Urine

Sutherland (1954) claims to find an increased urinary excretion of calcium in nearly 60% of his cases of renal calculi, but, since his diet was higher in calcium content than is usual in such studies, his figures are not directly comparable with those of other workers. Cottet & Vittu (1955) also found an increased urinary excretion of calcium in a high proportion of patients with renal calculus, but they made no attempt to standardize the calcium intake during their study. One of us has investigated this point in a consecutive series of patients attending a clinic for stone at the Royal Victoria Hospital, Belfast. Using a standard diet containing 154 mg. of calcium per 24 hours, we find that normal control subjects excrete from 48 to 148 mg. of calcium per 24 hours (mean 83 mg., S.D. 32 mg.). The urinary excretion of calcium has been estimated in 73 patients suffering from stone, who were on the same diet, and has been found in 30% of them to be greater than the mean plus twice the S.D. of the controls and can thus be considered to be increased. This figure includes six patients who were found to have hyperparathyroidism. However, the presence or absence of calculi does not appear to be solely related to hypercalciuria, for many patients excrete large quantities of calcium in their urine over long periods without stone formation. For example, marked hypercalciuria occurs in the Fanconi syndrome without calculus formation, and 20% of Hellström's patients with hyperparathyroidism did not have calculi (Hellström, 1955). Conversely, in our experience many patients who repeatedly form calculi do not excrete calcium in increased amounts. Despite this there would appear to be a relationship between hypercalciuria and stone formation, and in every case of renal calculus one should consider the possibility of hyperparathyroidism with its accompanying hypercalciuria.

The diagnosis of hyperparathyroidism may be difficult. The classic case with hypercalcaemia, hypercalciuria, hypophosphataemia and associated bone disease presents no difficulty. However, we have found that the hypercalcaemia fluctuates and may easily be missed if the serum calcium is estimated only once or twice, and in any case may be not much above 11 mg./100 ml. We estimate the serum calcium level on at least four occasions in each patient. The hypophosphataemia is not always present and tends to disappear with the onset of renal failure. The renal clearance of phosphate, or more exactly the ratio of the renal clearance of phosphate to the glomerular filtration rate, which is a measure of the proportion of phosphate reabsorbed by the renal tubules, has been suggested as being helpful in the diagnosis of hyperparathyroidism (Nordin, personal communication, 1955). This ratio must be considered in relation to the blood level of inorganic phosphate. The normal limits are shown in fig. 1. We have examined 103 patients in this way and, up to the present, we have not been impressed with this as a completely satisfactory test of parathyroid function. The ratio of phosphate to inulin clearance has been found to be raised in approximately 10% of patients with renal calculi in whom there was no other suspicion of hyperparathyroidism, and we have recently observed two patients without raised ratios, from whom parathyroid adenomas were removed.

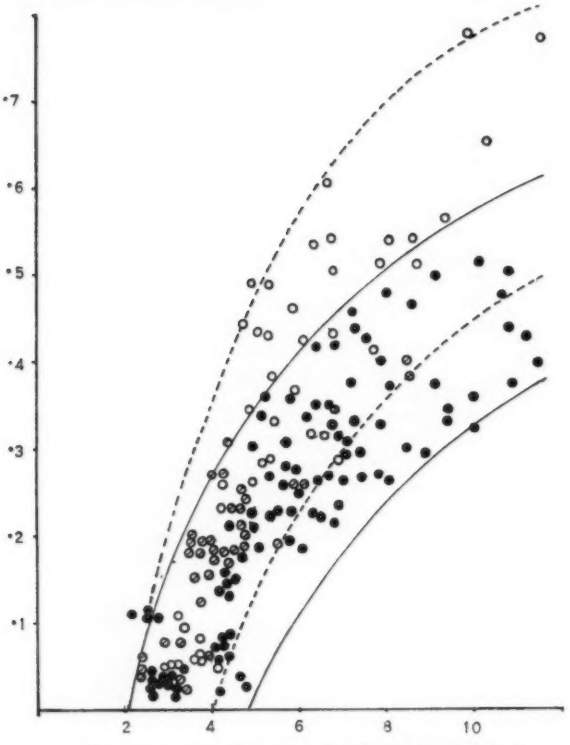
Anderson, Harper, Dent & Philpot (1954) report that the hypercalcaemia and hypercalciuria of sarcoidosis is reduced by treatment with cortisone, and that they did not find any such reduction in a single case of hyperparathyroidism. We also have not observed any reduction in the hypercalcaemia and hypercalciuria of hyperparathyroidism following administration of 200 mg. of cortisone daily for six days.

Indeed we have found no single test of parathyroid function that is satisfactory. As already stated, both hypercalcaemia and hypercalciuria may vary or even be absent. Of 76 patients who have been examined by means of calcium balances and serum calcium estimations, and on whom clearances of phosphate and inulin have been determined, 28 have been selected for exploration for parathyroid tumours on the basis of having two or more of the three tests positive. To date, six tumours and four glands with histological hyperplasia have been found in 15 cases operated on, but results show that complete reliance cannot be placed on any one test.

b. Urinary Infection

Urinary infection frequently accompanies renal calculi, and infections, especially with urea-splitting organisms, are

FIG. 1. RELATIONSHIP BETWEEN SERUM INORGANIC PHOSPHATE AND THE RATIO: RENAL CLEARANCE OF PHOSPHATE TO GLOMERULAR FILTRATION RATE (MEASURED BY CREATININE OR INULIN CLEARANCE)



Abscissae: serum inorganic phosphorus (mg./100 ml.)
Ordinates: ratio of urinary clearances of phosphate, and creatinine or inulin

- ● normal subjects (Lambert, van Kessel & Leplat, 1947)
- ⊙ normal subjects (Milne, 1951)
- ○ normal subjects (McGeown, unpublished data)

The continuous lines include the data of Lambert et *al.* and Milne; the dotted lines include the data of McGeown.

often regarded as being of great importance in the formation of stone. Urea-splitting organisms increase the alkalinity of the urine, which would increase the tendency to precipitation of calcium phosphate and magnesium ammonium phosphate. Hellström (1938), who considers that masses of bacteria can form nuclei around which calcium salts may be precipitated, claims to have identified bacteria in the centre of many stones. However, many cases of renal calculi occur in the absence of urinary infection, and even patients who have had calculi many times often have sterile urine on every occasion when it has been examined. Another fact which suggests that urinary infection does not play an essential part in the pathogenesis of calculus is that pyelonephritis is a disease more commonly found at post-mortem examination than is calculus and, although similar organisms are found in both conditions, many patients with chronic urinary infection never develop calculi. There is then no direct evidence that infection plays any essential part in the initiation of calculi, although it seems probable that it may encourage the growth of calculi in those already predisposed to them.

c. Urinary Stasis

It is obvious that obstruction to the outflow of urine would favour the retention of any minute fragment of inorganic or organic material which might form a nidus around which further deposition of salts might occur to form a macroscopic calculus. However, few cases of renal calculus are associated with obstructive lesions of the urinary tract, probably because the urine from an obstructed kidney is dilute (Woodside, personal communication, 1954).

d. Vitamin A Deficiency

Vitamin A deficiency has often been evoked as a cause for calculus formation, mainly because diets deficient in vitamin A have been used for the experimental production of calculi in rats (Higgins, 1933, 1935). Calculi can be formed in rats on diets which contain ample amounts of vitamin A. The relationship of these findings to the human disease is uncertain, but the hyperkeratosis which is present in the experimental avitaminosis is not a feature of the human kidneys containing calculi. Several series of cases have been surveyed for evidence of vitamin A deficiency, and usually no evidence of this is found (Council of Pharmacy and Chemistry, 1935).

e. Randall's Plaques

Randall (1939) described the deposition of calcium in the basement membrane of the collecting tubules and interstitial tissue just beneath the surface of the renal papillae. In some cases he was able to detect the beginning of calculus formation on such plaques which had eroded through to the surface, and he considered that these plaques were the starting point upon which calcium salts from the urine are deposited to form calculi. Other workers have been able to observe Randall's plaques, but these are often present without calculus formation, and cannot be detected in all kidneys which contain calculi. Vermooten (1941) observed Randall's plaques in the kidneys of Bantu negroes of South Africa, although renal calculi are very rare in this race. The nature of the relationship of these plaques to renal calculus remains unknown.

f. Microliths

Anderson & McDonald (1946) found microscopic calculi in normal kidneys and in kidneys removed for tuberculosis, pyelonephritis, hydronephrosis or calculus. These appeared to begin as little flecks of calcium in the interstitial tissue of the kidney, which were engulfed by macrophages, several of which coalesced together to form little " droplets of calcium ", and eventually plaques were formed. Sometimes these plaques ulcerated through to the surface of a papilla and could form the nidus for calculus formation. These tiny calculi were present in all kidneys from persons above nine years of age. Their relationship to the formation of calculi remains unknown, but they form the basis of an hypothesis to explain the pathogenesis of calculus, recently put forward by Carr (1954).

g. Hydrogen-Ion Concentration of Urine

Calcium phosphate and magnesium ammonium phosphate become more insoluble as the pH of the solution approaches

neutrality and the alkaline range, and therefore the pH of the urine might be expected to influence stone formation. The pH of voided urine is not necessarily the same as that of the urine in the renal calices and pelvis where calculus formation commences, and is probably different if infection is present. Calcium oxalate calculi may be found over the whole extent of the physiological range of urine pH. It has often been shown that urine is supersaturated with calcium salts when its pH is above 5.5, that is to say, that considerably larger amounts of calcium are held in solution than would be found in simple aqueous solution of calcium phosphate. In most specimens of normal urine, calcium does not precipitate as phosphate until the pH of the urine reaches 7.0 or higher, but in the urine of calculus-forming patients it precipitates between pH 6.0 and 7.0 (McGeown, 1955).

2. Current Theories of Calculus Formation

The current theories of calculus formation may be considered under three headings:

i. Those which postulate a deficiency of some substance or substances which in normal urine hold calcium in solution by the formation of a soluble complex, or by the adsorption of calcium.

ii. Those which postulate the presence of some substance which enhances the precipitation of calcium salts in the urine.

iii. A theory which postulates that calculus formation is due to a disturbance of the mechanism which normally rids the kidney of the microliths described by Anderson & McDonald (1946).

a. Deficiency of some Substance or Substances in Urine, which Normally Prevent Precipitation of Calcium

Many of the observed facts of calculus formation could be explained by such a deficiency. The tendency for calculi to recur in the same individual could be explained by the continuation of the original deficiency. The occurrence of calculi in some—but not all—patients who excrete abnormally large amounts of calcium in the urine could be explained by a relative deficiency of the hypothetical substance. Urinary infection could be regarded as a secondary occurrence, although it might play a part by bacterial destruction of the postulated substance which maintains calcium in solution. Medes (1935) reported that the presence of urea increased the solubility of calcium oxalate, but in physiological concentraf tions the urea in urine would not account for the amount o-calcium oxalate which could be precipitated in urine (Joly, 1939). Our experiments in which we have attempted to form a synthetic urine support Joly's view. Hammarsten (1936) considered that magnesium plays an important part in the pathogenesis of calculus but, although her experiments with rats on a diet low in magnesium are often quoted, further evidence has not appeared. Sabbatani (1901) observed that citrate bound calcium ions, to form a soluble complex. The urinary excretion of citrate was reported to be lowered in patients who form renal calculi, by Boothby & Adams (1934); Scott, Huggins & Selman (1943); Shorr, Almy, Sloan, Taussky & Toscani (1942). However, the urinary citrate values for both the normal controls and the patients with calculus were spread over a wide range although the average value was lower in the patients. Conway, Maitland & Rennie (1949) also estimated the urine citrate in normal individuals and in patients with calculus, and were unable to detect any difference unless the urine was infected. They were also able to show that *Escherichia coli*, *Streptococcus faecalis* and *Pseudomonas pyocyanea* destroy citrate. The citrate theory of calculus formation has recently been brought forward again in experiments by Harrison & Harrison (1955) in which the administration of a carbonic anhydrase inhibitor decreased the urine citrate and caused renal calcification in rats. Their experiments are open to the criticism that the increased alkalinity of the urine caused by carbonic anhydrase inhibitors may be as important as the reduction in urine citrate, or more important. It is difficult to devise experiments which would separate the two effects of carbonic anhydrase inhibitors, since in other conditions in which the urine is alkaline the urinary citrate is increased (Kuyper & Mattill, 1933; Boothby & Adams, 1934; Sherman, Mendel & Smith, 1936).

Joly (1939) thought that the urinary colloids were of great importance in the pathogenesis of calculi, the urinary crystalloids being held in solution by adsorption by the colloid particles. More recently Butt, Hauser & Seifter (1952) based their theory of the pathogenesis of calculi on a deficiency of protective colloid in the urine. They reported very good results in the prevention of recurrence of calculi by the injection of hyaluronidase, by which they hoped to increase the excretion of hyaluronic acid in the urine. This theory presupposes that hyaluronic acid or some other hyaluronidase substrate is a constituent of normal urine, but McGeown (1955) has been unable to find evidence of this. Smiddy (1954) has been unable to detect any change in the urine surface tension after the injection of hyaluronidase in normal individuals. Numerous other reports have followed on the use of hyaluronidase for the prevention of calculi but other workers have not been able to repeat the good results of Butt and his co-workers. It seems quite likely that the urinary colloids may play some part in holding calcium in solution in urine, but as yet direct evidence of this has not been produced.

Mandl, Grauer & Neuberg (1953) reported that several naturally occurring organic acids are capable of holding calcium carbonate and calcium phosphate in solution. These included galacturonic, ascorbic, glucuronic and gluconic acids. Acting on this suggestion, Prien & Walker (1955) have administered acetylsalicylic acid to patients who formed recurrent calculi. The urinary glucuronic acid was increased and the clinical results over a period of a year were good. These workers also made use of a high-fluid, low-calcium diet in these patients. It has not been suggested that stone formation is accompanied by reduced excretion of glucuronic acid.

In 1920 King & Palmer formed a crystalline addition compound between calcium chloride and L-glycine. Klement & Weber (1941) found that hydroxyapatite was more soluble in glycine and alanine solutions than in water. Over 6 g. of amino acids are excreted in the urine during 24 hours (King, 1951). It seems possible that these amino acids may help to hold calcium in solution, by the formation of soluble chelates. This has prompted us to look for abnormalities of amino-acid excretion in the urine. Some of our patients excrete reduced amounts of amino acids, while others show abnormal patterns of excretion. Experiments on rats have shown that the administration of glutamic acid will prevent calculus formation. These experiments will be reported in detail elsewhere. They raise the possibility that calculus formation may be due to an abnormality of tubular function.

b. The Presence of some Substance in the Urine, which Enhances the Precipitation of Calcium Salts

The chief exponents of this view are Boyce, Garvey & Norfleet (1954, 1955). They have demonstrated increased urinary excretion of mucoprotein in patients with renal stones, and a particularly large increase was found in cases of functioning parathyroid adenoma. This mucoprotein appears to be similar in its properties to that excreted by normal individuals. When isolated from the urine of patients with calculi, it was found to bind calcium to form an insoluble complex. When the incubating solution contained phosphate, a progressive formation of crystalline calcium phosphate followed the appearance of the calcium mucoprotein precipitate. These workers excluded cases showing symptoms or signs of acute urinary infection, but they do not state that infection was absent. An increased amount of mucopolysaccharide around and in cells of the renal tubules has been demonstrated by Baker & Sison (1954) and Baker & Connelly (1956), and this may lend support to the theory of Boyce and his co-workers. This hypothesis raised the interesting possibility that calculus formation may be a disorder of mucopolysaccharide metabolism, and it would be in line with the work of Sobel (1955) on the mechanism of calcification of bone. The matrix of calculi appears to be composed of mucopolysaccharides (Howard, 1954), but it is not yet proved that the excess excretion of mucoprotein is a primary rather than a secondary occurrence, although it persisted after the removal of all visible calculi (Boyce *et al.* 1954). It is difficult to explain the fact that it is raised in parathyroid adenoma, yet all such patients do not form calculi.

c. A Disturbance in the Mechanism which Normally Rids the Kidney of Precipitated Calcium

Anderson & McDonald (1946) found minute deposits of calcium in all kidneys, whether these contained calculi or not. Carr (1954) has been able to confirm these results by using a radiographic technique, and from his observations concluded that the deposits are situated in the renal lymphatics. By micro-*x*-ray diffraction analysis the microscopic calculi were shown to be identical with macroscopic calculi and with similar bodies in the perirenal fat, also presumably within lymphatics. Carr thinks that these minute deposits of calcium can progress to form calculi when the renal lymphatic system becomes overloaded with an excessive number of them, or when the renal lymphatic system is impaired by previous inflammation, or possibly when there is a deficiency of protective colloids.

The numerous hypotheses of the pathogenesis of calculi, for most of which there is at least some evidence, may mean that there is no single explanation for the formation of calculi. Calculus formation is probably prevented in the normal individual by a balance between the factors which tend to cause calcium to precipitate and those which tend to hold it in solution. The part played by the substances which form the organic matrix of calculi is as yet unknown, but the work of Boyce and his colleagues suggests that the matrix substance may be important. The volume of urine relative to the amount of calcium excreted must be important. The calcium content of the diet may also be a determining factor, although the urinary calcium is not directly proportional to that of the diet, as there are many factors which are known to affect both the absorption of calcium from the intestine and its excretion in the urine. In this connexion it is interesting to note that the calcium content of the diet of most people is probably greatly higher than it was before the practice of calcium fortification of flour was adopted.

At present we can influence calculus formation in the patient in two ways: firstly, by attempting to decrease the urinary excretion of calcium, and secondly, by decreasing the concentration of the urinary calcium by means of a high fluid intake. The first of these may be difficult to achieve, except in patients in whom a diagnosis of hyperparathyroidism can be confirmed. We encourage patients to adopt a high fluid intake, especially in the evening, in order to prevent a concentrated night urine. We try to exclude hyperparathyroidism as a factor in the excretion of calcium. We try to eradicate urinary infection, not because we think it plays an important part in the pathogenesis of calculi, but because of the damage to renal function caused by chronic pyelonephritis.

3. Summary

Conditions which are frequently associated with calculus formation are reviewed. It is significant that stones can occur in the absence of all of them. The part played by these conditions is probably of secondary importance. The current hypothesis that stone formation is due to a deficiency of some substance which holds calcium in solution in normal urine, or to an excess of some substance with which calcium forms an insoluble complex, cannot be regarded as proved. The prophylaxis against recurrences of calculi after their surgical removal rests upon the maintenance of a high fluid intake, and the treatment of coexisting urinary infection.

ACKNOWLEDGEMENTS

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AZOTAEMIC RENAL OSTEODYSTROPHY

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1 Historical review: a background to contemporary confusion
2 The natural history of renal osteodystrophy: a personal concept
3 Rickets and osteomalacia
4 Renal osteitis fibrosa
5 Osteosclerosis and osteoporosis
6 Treatment of azotaemic renal osteodystrophy
References

The term " renal osteodystrophy " was introduced by Liu & Chu (1943) to describe collectively the various skeletal complications of chronic renal disease. In the present review, discussion will be restricted to the osseous complications of renal disease with retention of nitrogen; but it should be remembered that rickets or osteomalacia can result occasionally from disturbances of the homeostatic functions of the kidney in patients showing no evidence of renal excretory failure. The rare renal tubular disorders that may lead to defective mineralization of the skeleton have been discussed elsewhere in several comprehensive reviews (Albright, Burnett, Parson, Reifenstein & Roos, 1946; Jackson & Linder, 1953; Bickel, Smallwood, Smellie & Hickmans, 1952; Dent, 1952; Dent & Harris, 1956). It must be admitted at the outset that it is not known for certain why the presence of renal excretory failure should lead to the development of the gross skeletal abnormalities that are discussed subsequently. This simple fact serves as a crude point of distinction from the bone disorders caused by renal tubular disease.

The only biochemical abnormality that is constantly associated with azotaemic renal osteodystrophy is elevation of the blood level of non-protein nitrogen; a metabolic acidosis is almost always present, but it is not invariable. The serum level of inorganic phosphorus is usually elevated, but it may be normal or, infrequently, reduced; the serum calcium level may be normal, lowered or occasionally elevated. Elevation of the serum level of alkaline phosphatase is usually found when the osseous changes are extensive enough to have produced clinical symptoms.

1. Historical Review: a Background to Contemporary Confusion

The association of renal disease with late rickets was first recorded by Lucas (1883). Subsequently, the relation of kidney disease to disorders of the skeleton was studied extensively by clinicians in Great Britain, where the observations of Fletcher (1911), Barber (1913, 1918, 1920, 1921, 1933), Parsons (1927) and many others defined the principal clinical and radiological features of renal osteodystrophy as it occurs in children and adolescents. The terms " renal dwarfism ", " renal infantilism " and " renal rickets ", although obviously not invariably applicable, were apt in their original use. It was, moreover, fully appreciated that dietary deficiency of vitamin D played no part in the pathogenesis of the rachitic changes and that therapy with cod-liver oil was not curative (Parsons, 1927). With these reservations, the use of the word " rickets " to describe a characteristic disturbance of what has been called the " growth apparatus " (McLean & Urist, 1955) was completely justifiable. Many competent pathologists have shown that the histological lesion of the growth apparatus in renal rickets is essentially the same as that in nutritional rickets (Turnbull, cited by Hunter (1930); Gilmour, 1947; Follis, 1950; Baker, 1954).

In 1937, Albright, Drake & Sulkowitch described an adult patient with chronic azotaemic renal failure, metabolic acidosis, metastatic calcification, hyperplasia of the parathyroid glands and changes in the skeleton that were indistinguishable from the osteitis fibrosa of primary hyperparathyroidism. Simultaneously, Park & Eliot (1937) demonstrated the lesions of osteitis fibrosa in children with renal rickets. These American observations have so influenced subsequent thought on renal osteodystrophy that most contemporary accounts consider either renal osteitis fibrosa or renal hyperparathyroidism to be a suitably comprehensive description of the osseous disorders of chronic renal excretory failure. It has even been asserted that renal rickets " ' is not rickets at all but osteitis fibrosa generalisata ' " (Albright & Reifenstein, 1948). That this is categorically incorrect was proved by the histological studies of Gilmour (1947) and of Follis (1950), and it is at variance with our own

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experience. Moreover, as will be shown subsequently, renal osteitis fibrosa is not the only osseous lesion developing in adults with chronic azotaemic renal failure; such patients may develop a florid osteomalacia that is the adult counterpart of renal rickets.

It has been emphasized, both by Follis & Jackson (1943) and by Gilmour (1947), that relatively few worth-while pathological studies were made of the bones in the earlier, individually recorded cases of renal osteodystrophy. This is undoubtedly true, and consequently no attempt will be made here to review the literature of the disorder. The present description will summarize briefly our experience of approximately 25 azotaemic patients with bone disease observed in Manchester during the past eight years. A more detailed account of the clinical, radiological, metabolic and pathological features will appear elsewhere (Ball, Lumb & Stanbury, to be published).

2. The Natural History of Renal Osteodystrophy: a Personal Concept

Bone disease in patients with renal failure may be present without producing significant symptoms; or, as we have often observed, symptoms may be present literally for years without their significance being appreciated. It is thus possible to miss completely the earliest stages of the bone disease. Secondly, in a chronic disorder of slow evolution, observations of short duration pertain only to the contemporary state and provide little insight into past events, or those probable in the future; this is equally true of clinical, radiological and metabolic studies. Ideally, therefore, in order to construct a picture of the natural history of azotaemic renal osteodystrophy, it would be necessary to follow the clinical and metabolic changes throughout the whole illness, and correlate these with the structural changes occurring in the bones. In practice this is impossible. In our own studies, my colleagues and I have compromised by observing a relatively large number of patients, in some of whom we have been able to trace the evolution of the disease over the course of many years. Radiology and bone biopsy have been used as complementary to clinical and metabolic observations and, whenever possible, a more detailed histological analysis has been made at autopsy.

From this composite experience we would propose the following hypothesis: " Azotaemic renal osteodystrophy is a disorder of chronic course; it may consist of several distinct components of probably differing pathogenesis, which are active in varying degree in different patients; these individual components may occur alone or variously admixed; and, in the course of time, one pattern of bone disease may become transformed into another ". A somewhat similar concept was suggested by Berner (1944) and by Gilmour (1947).

Among a group of patients with azotaemic renal osteodystrophy, one may detect disturbances of almost all the biological processes that are known to involve bone as a tissue and bones as units of structure. Arrest of endochondral growth at the epiphysis may lead to dwarfism or, if temporary, it may leave its mark in the transverse lines of arrested growth above the metaphysis (see Plate V, fig. B; Harris, 1933; Park & Richter, 1953). Defective deposition of bone mineral may give rise to rickets in the child (Plate V, fig. B) or to a painful osteomalacia in an adult of any age (Plate V, figs. Ca, Cb). The integrated processes responsible for remodelling of the long bones (Sissons, 1956) may fail, with resulting distortion in the metaphyseal regions, or inappropriate distribution of bone trabeculae in relation to directional stresses. The processes producing reabsorption of bone may be stimulated to excessive activity; trabeculae are replaced by fibrous tissue containing osteoclasts (Plate VIII, fig. J), the skeleton is reduced in density (Plate VI, fig. D; Plate VII, fig. G) and sometimes cysts are formed (Plate V, fig. Ab). Bone mineral that is released from the skeleton may be deposited in soft tissues throughout the body (Plate VIII, fig. K). Reactive formation of new bone accompanies bone destruction (Plate VI, fig. D; Plate VII, fig. G); the new bone may be formed in haversian systems and it can simulate Paget's disease in both radiological and histological appearance (Ginzler & Jaffe, 1941), or the normal lamellar bone may be replaced largely by woven bone (Plate VIII, fig. J). Occasionally, new formation of lamellar bone amounting to osteosclerosis may occur without apparent relation to associated bone destruction (Crawford, Dent, Lucas & Martin, 1954) or there may be an exuberant and unexplained accretion of periosteal bone (Claireaux, 1953; see Plate VII, fig. Fb). Not infrequently, these various pathological lesions occur together in an apparently illogical fashion; as when osteosclerosis in one part of the skeleton is associated with active rickets elsewhere (Claireaux, 1953), or when the calvarium is enormously thickened and its tables obliterated, while in the adjacent basiocciput the bone appears to be normal and tabular distinction is clearly preserved (personal observations). The complexity of the problem is illustrated nicely in the contrasting appearances of the two vertebrae shown in Plate V, fig. A.

It is thus not possible to describe azotaemic renal osteodystrophy as a simple or single pathological entity; it can best be regarded as an extraordinarily intricate series of reactions of the skeleton to the presence of renal insufficiency. Of the many components in this skeletal spectrum, rickets (osteomalacia), osteitis fibrosa and osteosclerosis invite further discussion because of their relevance to general problems of metabolic bone disease.

3. Rickets and Osteomalacia

The patient with renal rickets may exhibit any of the clinical abnormalities of the skeleton that are seen in late rickets of nutritional origin. Comprehensive clinical descriptions are available in the classical papers by Barber and by Parsons, and little would be gained by recapitulation here. The osteopathology was described in detail by Gilmour (1947). As in other forms of rickets, the development of renal rickets is contingent upon the occurrence of endochondral growth of bone (Plate V, fig. B), and arrest of growth may account for apparent healing in certain circumstances. Established renal rickets is uninfluenced by physiological amounts of vitamin D but, like the familial " vitamin D resistant rickets ", it can be healed therapeutically by very large doses of the vitamin D steroids; and we have seen such healing occur in some patients in spite of persisting metabolic acidosis.

One factor that may have facilitated ready acceptance of the notion that renal rickets should be regarded as renal osteitis fibrosa was that no adult counterpart in azotaemic renal osteomalacia had then been recognized clinically. Histopathological proof that osteomalacia occurs commonly in azotaemic patients was provided by the autopsy studies of

AZOTAEMIC RENAL OSTEODYSTROPHY

(FIGS. A—K)

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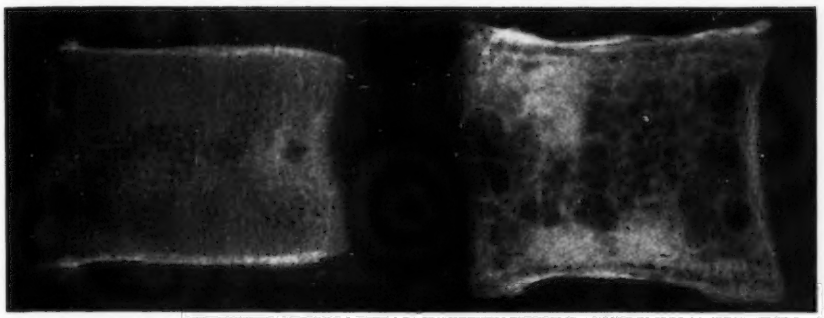


a b

Fig. A. The diversity of the osseous changes in chronic azotaemic renal failure

Radiographs of hemisected vertebral bodies from two patients dying of uraemia; in each patient the primary renal lesion was bilateral hypoplasia and each had developed secondary pyelonephritis

a: the vertebra showed only the histological changes of severe osteomalacia; age at death, 18 years. (Same patient as in fig. **B**)

b: this vertebra shows cyst formation resulting from renal hyperparathyroidism. There is also a subchondral condensation of the spongiosa, the new bone formation in this situation having produced an appearance of osteosclerosis. The marked mineral density may be related to previous therapy with calciferol. Eight years earlier this same patient had exhibited clinical and radiological evidence of rickets. (Same patient as in figs. **Db, Fa, H, J, K**)

Fig. B. The development of rickets in a " renal dwarf "

a: aged 13 years; great retardation of growth but neither clinical nor radiological evidence of other bone changes. (The patient was one of a pair of identical twins, see Milne, 1951.)

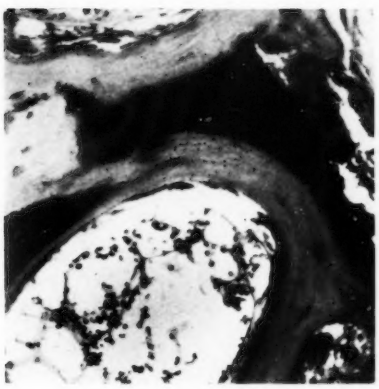
b: aged 16 years; evidence of growth, with early rachitic changes at the ulnar metaphysis

c: aged 18 years; the appearances at death, the patient having suffered severe bone pain for two years

a b c

Note that the radiological appearances are indistinguishable from those of late nutritional rickets or rickets due to steatorrhoea or to renal tubular disease. There is no evidence of the metaphyseal erosion commonly seen in advanced renal rickets (Teall, 1928). A line of arrested growth is indicated by the arrow in **c**. (Same patient as in fig. **Aa**)

The histological changes in the growth apparatus were those of classical rickets, but changes typical of osteitis fibrosa were also present in the metaphyseal area



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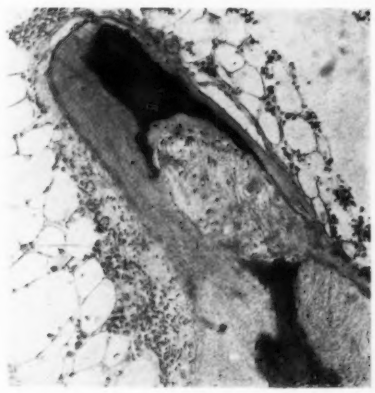


b

Fig. C. Sections of undecalcified cancellous bone obtained from the iliac crest by biopsy (courtesy of Dr J. Ball)

These two examples are chosen to illustrate the advanced age at which clinically obvious osteomalacia may develop

a: male, aged 63 years; severe bone pain for three years; hypertensive, blood urea 100 mg./100 ml. Complete symptomatic relief following administration of calciferol in a dose of 6.25 mg. (250,000 i.u.) daily by mouth

The black area of calcified bone is ensheathed by a deep seam of osteoid in which no mineral is demonstrable. Stain: von Kossa and safranine

b: female, aged 77 years; normotensive, blood urea 140 mg./100 ml.; atrophic pyelonephritis. Original clinical diagnosis: pernicious anaemia with osteoarthritis. Symptomatic relief from calciferol therapy; early death from miliary tuberculosis

The histological appearances are similar to those in (a) with the addition of the changes of osteitis fibrosa. The darker stained area of calcified bone shows the dissecting trabecular reabsorption characteristic of osteitis fibrosa. Such lesions were infrequent and osteomalacia was the predominant histological feature. Stain: haemalum and eosin

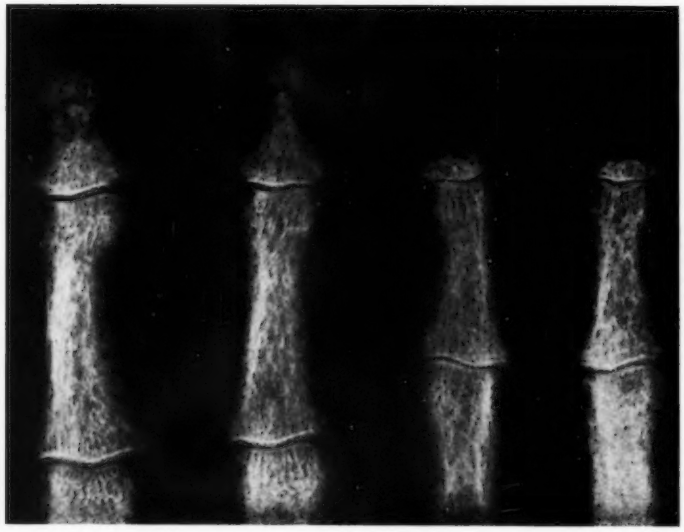


FIG. D

a

b

Fig. D. Radiological appearances of renal hyperparathyroidism

a: progressive destruction of the terminal phalanges illustrated in different fingers from the same patient. This change may be associated with a clinically detectable foreshortening of the distal phalanx and an appearance of " pseudo-clubbing ". Note, especially in the middle phalanges, the scalloped erosions of the periosteal surface of the cortex and the almost complete loss of organized cortical structure. Although the changes are predominantly destructive, there is abundant evidence of reactive formation of new bone

b: in this lateral radiograph it can be seen that the distal phalanx has been eroded down to the interphalangeal joint. There is a mass of metastatic calcification at the site of the original distal phalanx; this probably developed as a result of therapy with calciferol

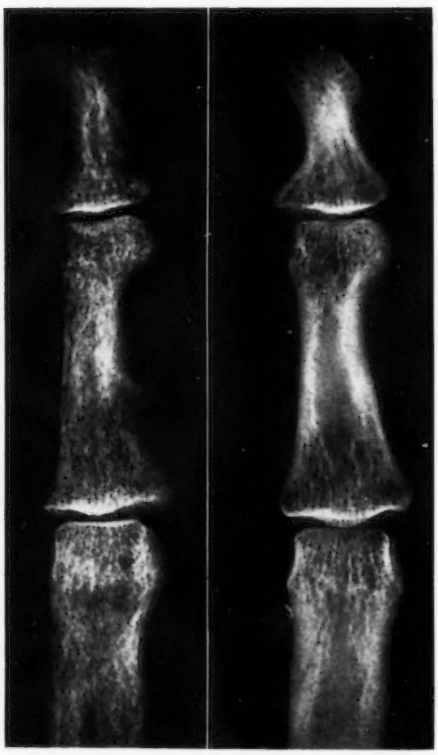
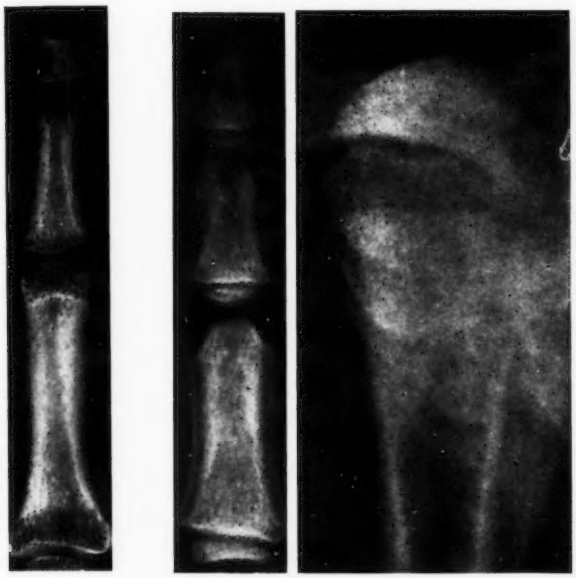


FIG. E

Fig. E. Radiological lesions of primary hyperparathyroidism and their healing following removal of a parathyroid adenoma

For comparison with fig. **D** and fig. **G**. Note that before operation the phalanges show extensive erosion together with some evidence of reactive formation of new bone. There is complete reconstruction of bone architecture following parathyroidectomy

FIG. F



a b

Fig. F. Osteosclerotic changes in azotaemic renal osteodystrophy

a: greatly thickened and relatively structureless phalangeal cortices in a patient over-treated with calciferol. (Same patient as in figs. **Ab, Db, H, J, K**)

b: spontaneous accretion of periosteal bone. The changes exemplified here in a finger and a humerus were present in almost all bones of the skeleton. (Patient of Claireaux, 1953)

The radiograph of the humerus was obtained from a routine chest film, and the outline of the humerus is partly obscured by the scapula

Fig. G. Healing of renal osteitis fibrosa after therapy with dihydrotachysterol

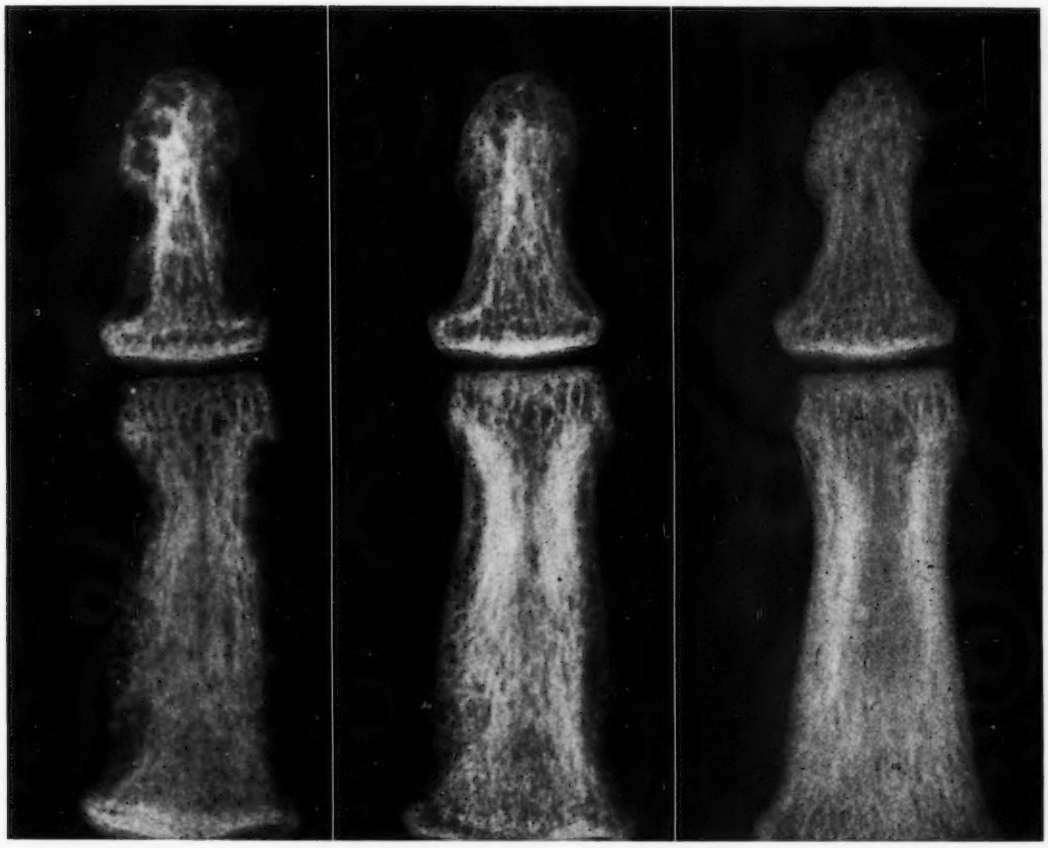
a: before treatment; extensive erosion of the periosteal surfaces together with reactive formation of calcified subperiosteal bone

b: an early effect of treatment appears to be a more complete ensheathing of the original cortex by calcified new bone

c: a new and distinct cortex has formed and some remodelling has taken place

Dihydrotachysterol therapy was controlled by metabolic balance studies. (Full details of the metabolic investigations on this patient will be published subsequently by Professor C. E. Dent, Department of Medicine, University College Hospital, London.)

FIG. G



a b c

Fig. H. Parathyroid hyperplasia in azotaemic renal osteodystrophy

Dissection of the posterior aspects of the thyroid gland and oesophagus to reveal the four enormous parathyroid glands. The parathyroid glands (total weight, 7.1 g.) have been displaced from their original positions

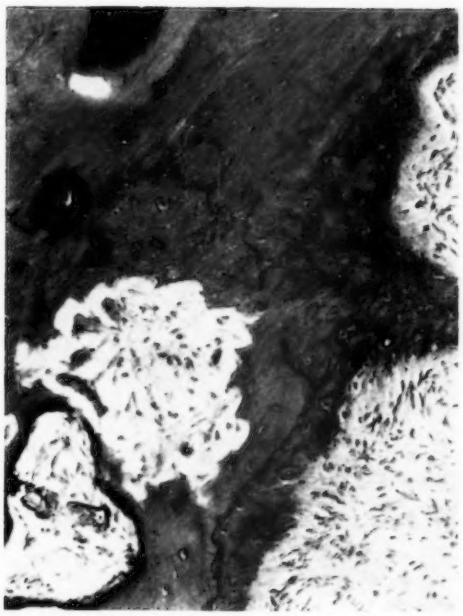


Fig. J. Osteitis fibrosa in bone rendered sclerotic after calciferol therapy

Imagine a line drawn diagonally from the bottom left to top right corner; above the diagonal, lamellar bone is subject to lacunar absorption; below and to the right, woven bone merges with uncalcified connective tissue. (Same patient as in figs. **Aa, Db, Fa, H, K**) Decalcified preparations. Stain: haematoxylin and eosin

FIG. K a

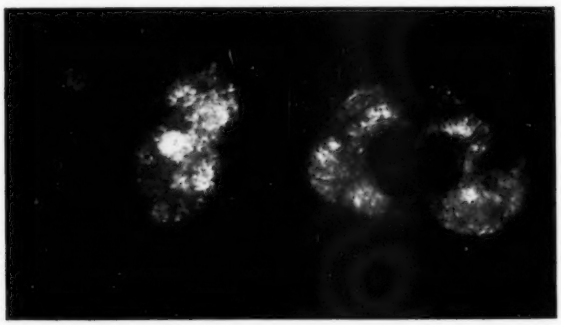


FIG. K b

Fig. K. Hazards attending uncontrolled recalcification therapy in renal osteitis fibrosa

For almost a year, this patient was given very large doses of calciferol by her doctor. Her bones became very dense (figs. **Ab, Fa**) and she had no skeletal symptoms. It was evident that calciferol had forced a greater retention of calcium than could be accommodated even in the osteosclerotic skeleton

a: an enormous area of metastatic calcification involving the thighs, buttocks, perineum, lateral pelvic and lateral abdominal walls. Note the radiologically dense vertebrae and the thick femoral cortices

b: post-mortem radiograph of the kidneys from the same patient, showing extensive mineral deposits

Follis & Jackson (1943) and Berner (1944), and the extensive lesions sometimes found by these observers suggested that they could have given rise to symptoms during life. We have found that, by paying careful attention to the complaints of chronically uraemic patients, one can sometimes distinguish the symptoms of generalized bone disease. In one such patient (aged 22 years; blood urea, 190 mg./100 ml.) typical spontaneous fractures of several ribs were found shortly after her first pregnancy. She received 1.25 mg. (50,000 i.u.) of calciferol daily together with calcium lactate for six months, until her death following a second abortion. At autopsy, her bones showed florid osteomalacia; Professor S. L. Baker, commenting on a section of rib, stated that "the osteoid tissue exceeds in amount the calcified bone". This constituted firm clinical and pathological proof both of the occurrence of osteomalacia and of the fact that the osteomalacia is "vitamin D resistant". Subsequently, in other patients, we have verified our clinical suspicions by histological examination of undecalcified bone (Ball, 1957) obtained from the iliac crest by biopsy, and have been able to provide symptomatic relief and presumptive cure by very much larger doses of calciferol (Plate V, fig. C). It must be emphasized, however, that there may be no radiological evidence of bone disease in these patients; the patient's symptoms alone must provide the stimulus to further search, and the minor discomfort of bone biopsy is fully justified by the prospect of symptomatic relief.

It is the author's belief that the rickets or osteomalacia of chronic uraemia results from an acquired insensitivity to the action of vitamin D. There is a failure of calcium absorption from the gut, as in the familial form of vitamin D resistance (Dent & Harris, 1956), and phosphorus absorption is also defective, so that the patient is in moderate negative external balance for these minerals when receiving an average normal diet (Liu & Chu, 1943). Healing of the rickets following therapy with calciferol or dihydrotachysterol is associated with increased intestinal absorption and with a strongly positive calcium and phosphorus balance. The nature and immediate cause of the vitamin D resistance are not apparent, but it is our clinical impression that it cannot be ascribed to the acidosis of renal failure. The solution of this fascinating problem should ultimately contribute greatly to the understanding of the actions of vitamin D.

4. Renal Osteitis Fibrosa

As with rickets and osteomalacia, renal osteitis fibrosa may be present without producing clinical or radiological signs; but, when advanced, its effects may be indistinguishable from those seen in primary hyperparathyroidism (Plate VI, figs. D, E; Plate VII, fig. G). Greater or lesser degrees of osteitis fibrosa may be present in association with rickets (see legend, Plate V, fig. B) or osteomalacia (Plate V, fig. Cb); but this occurrence should probably be interpreted as the simultaneous presence of two distinct and largely independent pathological processes. It is the presence of osteitis fibrosa that accounts for the metaphyseal erosions (the "rotting post" of Teall (1928)) in renal rickets and is largely responsible for the so-called "epiphyseal slipping" or metaphyseal fractures.

Although there is no dispute concerning the occurrence of osteitis fibrosa as a component of renal osteodystrophy, there is a general reluctance to accept parathyroid hyperfunction (see Plate VIII, fig. H) as the cause for this tissue change. In my opinion, the tendency to exonerate the parathyroid glands is at variance with the evidence; and the view that renal osteitis fibrosa is a result of the metabolic acidosis of glomerular insufficiency cannot be supported. The most conclusive evidence of the active role played by parathyroid hyperplasia is provided by the experience of Dreskin & Fox (1950), in whose patient the hyperplasia involved predominantly one parathyroid gland. Surgical removal of this "tumour", which had the typical histological appearances of hyperplasia, was followed by the development of tetany, resolution of areas of metastatic calcification and both radiological and histological evidence of bone healing. Gilmour (1947), in his series of autopsies, considered the amount of osteitis fibrosa to be roughly proportionate to the weight of parathyroid parenchyma; and, in chronic canine nephritis, Platt (1951a, 1951b) found much larger parathyroid glands when osteitis fibrosa and clinical osteodystrophy ("rubber jaw") were present. Finally, Eger (1941) found that the osteodystrophy that complicates uranium nephritis in the rat did not occur when the parathyroids were first removed.

Metastatic calcification in chronic renal failure is almost invariably associated with considerable parathyroid enlargement and extensive osseous involvement with the lesions of osteitis fibrosa (Herbert, Miller & Richardson, 1941; Gilmour, 1947); and it is generally assumed that the calcium and phosphate ions released from bone over-saturate the extracellular fluids and lead to the deposition of bone salt in the soft tissues. It is, however, apparent that a multiplicity of complex factors determine both the occurrence and the sites of pathological calcification (Hass, 1956). We have seen extensive calcinosis in patients with radiologically normal bones in which the lesions of osteitis fibrosa were minimal.

5. Osteosclerosis and Osteoporosis

Osteosclerosis, involving most of the skeleton, may follow as a consequence of excessive calciferol therapy in patients with renal osteitis fibrosa (Plates V & VII, figs. Ab, Fa). A reactive formation of new bone in the presence of osteitis fibrosa is the rule (Ginzler & Jaffe, 1941; Berner, 1944) and sometimes it is excessive, giving rise spontaneously to the radiological appearances of osteosclerosis. Areas of bone exhibit increased radiological density, often with a chalky appearance and loss of textural detail. Such changes may occur at the base of the skull (Claireaux, 1953), in the long bones about the metaphyses, on periosteal surfaces (fig. Fb) and in the upper and lower parts of the vertebral bodies (Ginzler & Jaffe, 1941; Crawford *et al.* 1954). The latter authors found vertebral osteosclerosis both in the presence and in the absence of osteitis fibrosa and apparent parathyroid hyperplasia. Berner (1944) and Hamperl & Wallis (1933) record individual instances of vertebral "densification" in which the tissue consisted largely of uncalcified osteoid. The significance of the rather rare florid osteosclerosis of renal osteodystrophy, and its relation to the osteosclerosis of idiopathic infantile hypercalcaemia, is at present obscure. In attempting to assess its significance, it should be remembered that a subchondral condensation of the spongiosa is often found in vertebrae affected with osteitis fibrosa due to primary hyperparathyroidism (Jaffe, 1933); its occurrence has been detected radiologically in this disease (Albright, Baird, Cope & Bloomberg, 1934). Apparent osteosclerosis in the metaphyseal areas has also occurred in primary hyperparathyroidism (Adam & Ritchie, 1954).

Osteoporosis, in the sense of a quantitatively inadequate formation of bone matrix (Albright & Reifenstein, 1948), has not been generally recognized as a component of renal osteodystrophy, although it was present in five of the younger patients of Berner (1944). It is thought to occur more frequently in children with nephrosis (Emerson & Beckman, 1945; Stanbury & Macaulay, 1957), but this belief is based on radiological appearances and, so far as we are aware, it has not yet been subject to histological proof.

6. Treatment of Azotaemic Renal Osteodystrophy

One cannot satisfactorily dispose of therapy within the confines of a short review. The treatment of rickets and osteomalacia with calciferol or dihydrotachysterol, although it can produce excellent clinical results, is potentially dangerous and should not be undertaken lightly. The very large doses of steroid (1.5–6.25 mg. daily) that are commonly required may readily produce the toxic effects of hypercalcaemia and metastatic calcification (Plate VIII, fig. K); and, in the individual patient, there is no means of foretelling the degree of vitamin D sensitivity. It has also been found during intensive therapy with vitamin D that the radiological appearances of renal osteitis fibrosa may change, in a manner that is strongly suggestive of healing (Plate VII, fig. G; and compare with fig. E). These radiological impressions of healing may, however, be illusory; for, even in densely sclerotic and heavily mineralized bone, we have found evidence of extremely active osteitis fibrosa (Plate VIII, fig. J). This occurrence underlines the especial dangers of undertaking recalcification therapy in patients with overt osteitis fibrosa; even though the greater part of the retained minerals is deposited in the skeleton, it is likely to be released therefrom by the continued activity of the osteolytic processes (see Plate VIII, fig. K).

Ideally, the use of vitamin D preparations in azotaemic patients should be controlled by metabolic balance study, which will furnish a fairly accurate measure of mineral retention and facilitate the regulation of dosage. Therapy without metabolic control is less dangerous in osteomalacia and rickets, where the skeleton contains large amounts of osteoid; but frequent biochemical examination of the blood should be made, and it is wise to be satisfied with symptomatic relief rather than to aim at the restoration of normal radiological appearances. Vitamin D steroids should not be given to patients in whom the predominant osseous lesion is renal osteitis fibrosa; even with full metabolic control, this would be little more than a hazardous therapeutic experiment.

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RENAL ANGIOGRAPHY

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1 Translumbar aortography
2 Retrograde femoral catheterization
3 Selective renal angiography
4 Contra-indications and complications
5 Indications
6 Conclusions
References

Radiographic demonstration of the renal arterial blood supply can be achieved by several different methods. The venous side of the renal circulation cannot be satisfactorily investigated by the methods to be discussed, but the capillary circulation can be assessed by the opacification of the kidney substance which occurs during the passage of injected contrast medium through the small arteries and the capillaries.

The first method to be used, by dos Santos, Lamas & Caldas (1929), was translumbar aortography. The second method, developed by Seldinger (1953), is known as retrograde femoral catheterization. With both these methods there are two serious disadvantages: firstly, large quantities of concentrated contrast medium must be injected, for example, 30 ml. of 70% diodone is commonly required; secondly, both kidneys are simultaneously flooded with the medium. These two factors may have been responsible for renal damage, and even death, in some of the recorded cases. Recent work in Sweden by Seldinger & Edholm (1956) and by Tillander (1956) has aimed at developing a more selective technique, so that small quantities of less concentrated contrast medium can be injected into the renal artery of the kidney under investigation. Although at present this procedure is complicated and requires considerable time and skill for its performance, it seems to us that the lessened risk of renal damage and the improved definition of the renal circulation, free from the confusion caused by contrast medium passing into arteries not of immediate interest, will make these newer methods potentially more valuable than the other two. For a brief historical review and description of the technique of translumbar aortography and retrograde femoral catheterization the reader is referred to the article by Riches & Whiteside (1956). The original papers by Seldinger and Tillander should be consulted for detailed accounts of the methods used by these authors. For the purpose of the present paper a brief description only of the techniques will be given.

Irrespective of the method of injection used, the radiographic procedure is the same. The patient lies on some form of tunnel placed on the *x*-ray table. This tunnel allows a number of *x*-ray cassettes to be slipped into position beneath the patient so that a series of radiographs can be taken at intervals. In the majority of cases three films suffice. One taken towards the end of the injection demonstrates the main arterial supply of the kidney: this film is known as a renal arteriogram. The second film, taken two seconds later, shows opacification of the kidney substance, and some of the smaller vessels are still visible. The opacification of the kidney substance is due to contrast medium in the capillaries and tubules: this film is known as an early nephrogram. The third film, exposed two seconds after the second, is the late nephrogram and is similar to the earlier one except that, should a tumour be present, the contrast medium will often tend to remain pooled in the tumour vessels.

The diagnostic features to be looked for are the size and distribution of the extra-renal and intra-renal arteries; the presence of abnormal arteries as, for example, in tumours or in angiomata; the displacement of the intra-renal, and sometimes the extra-renal, arteries by tumours or cysts; and, finally, in the nephrogram, the uniformity or otherwise of the opacification of the kidney substance and the relative rate of clearing of contrast medium from the kidney.

1. Translumbar Aortography

Although this procedure can be performed under basal anaesthesia, it is much more satisfactory to use general anaesthesia. When this is used the anaesthetist is required to arrest respiration temporarily while the radiographic exposures are being made so as to avoid loss of detail due to movement of the kidneys. The anaesthetized patient is placed prone on the radiographic tunnel and a needle approximately 6 in. (15 cm.) long, with internal diameter 16 standard wire gauge, is inserted approximately 4 in. (10 cm.) to the left of the spine of the second lumbar vertebra and directed medially and ventrally, with a cephalic tilt towards the body of the twelfth thoracic vertebra. When the needle is felt to strike bone, it is withdrawn slightly and the ventral tilt increased. It is again advanced in this new direction until the vertebra is again encountered. The process is repeated until the needle is felt to slip past the side of the vertebral body. At this stage the stillette is withdrawn and the needle advanced cautiously until blood is seen to drip steadily from the needle when the aorta is entered. A length of plastic tubing with a 20-ml. syringe joined by a Luer-Lok connexion is then attached to the needle, and normal saline injected. If the needle is correctly placed, no difficulty is experienced in injecting saline. A few rapid injections of 10–15 ml. are made and, if these prove satisfactory, the injection of the contrast medium can proceed immediately. If, after a trial injection, it is found that there is an increased resistance, or that the regurgitation of blood along the needle and tubing is slower than before, this indicates the need to readjust the needle before further injections are made. When the position of the needle is considered satisfactory, protective lead screens are brought into position to protect the operator and the radiographic cassettes from stray radiation. The injection is made rapidly, i.e. 30 ml. of 70% diodone should be injected in not more than four seconds. Some authors use compressed-air pumps or mechanically operated lever mechanisms to increase the pressure of the injection, but it is possible that these increase the risk of renal damage.

The first radiographic exposure is made in response to a call from the operator after approximately three-quarters of the contrast medium has been injected. The second exposure is made two seconds later; and the third, two seconds after this.

If the films are satisfactory the needle is withdrawn and the puncture wound sealed with collodion. If the films are unsatisfactory a second injection can be given, but there is some evidence that this increases the risk of renal damage.

In many cases it is desirable to make a single exposure after

the injection of a small quantity of contrast medium, such as 5 ml., to make quite certain that the needle point is just above the origin of the renal arteries. This will avoid the necessity of a second large injection should the original placing of the needle point be incorrect.

2. Retrograde Femoral Catheterization

This method, developed by Seldinger, aims at introducing a polythene catheter percutaneously. For this purpose he developed a special trocar, which is introduced into the femoral artery at the level of the inguinal ligament and in a retrograde direction. Local anaesthesia suffices. When the needle is correctly in position, as recognized by the free flow of blood from it, a guide wire is introduced via the lumen of the needle, which is then withdrawn over the guide wire which remains in the femoral artery. The catheter is then threaded on to the guide wire and pushed along until it finally enters the lumen of the artery through the original puncture. The guide wire can then be withdrawn and the catheter pushed gently upwards through the external and common iliac arteries into the aorta. When a sufficient length has been introduced, a small quantity of contrast medium is injected and a film is exposed at the appropriate time to demonstrate that the tip of the catheter lies correctly in relation to the renal arteries. A radio-opaque tip on the catheter assists in the correct placing of it. The injection and the timing of the films are the same as in the translumbar method.

Both these methods have certain advantages and some definite disadvantages. In both cases relatively large quantities of concentrated contrast medium are needed. Arteries, other than the renal arteries, are filled with contrast medium and slightly obscure the picture. Furthermore, both kidneys are flooded with contrast medium simultaneously and, should this produce renal damage, both kidneys will suffer.

The retrograde method is more difficult to perform, takes longer and is frequently impossible if the large arteries are tortuous, due to atheroma, or if there should be thrombosis present. On the other hand, once the catheter is in position, the rest of the procedure is simple and there is the advantage that the patient can be *x*-rayed in the supine and oblique positions; this brings the kidneys closer to the film and so tends to improve the radiographic detail.

The translumbar method is certainly simpler and quicker, but the exact placing of the tip of the needle in relation to the renal arteries is somewhat uncertain and a second or third puncture may be required in some cases.

3. Selective Renal Angiography

Seldinger and Tillander, working independently, have devised methods of ensuring that the contrast medium passes directly into the renal artery on the affected side. Seldinger uses a special catheter, introduced percutaneously as described above, and pushed in retrograde direction up the aorta a short distance above the origin of the renal arteries. The catheter contains a guide wire which, on being withdrawn a few centimetres, allows the tip of the catheter to curve laterally. When the whole catheter is then withdrawn slowly down the aorta, the curved tip will frequently be found to slip into the opening of the appropriate renal artery.

Tillander's method relies on the use of a strong magnetic field placed outside the body. He uses a catheter with several metallic segments at the tip joined together by ball-and-socket joints, thus allowing free movement in all directions of the tip. The tip of the catheter is first placed approximately in the position of the origin of the renal arteries, having been introduced in retrograde fashion as with Seldinger's catheter. The magnetic field is then used to direct the tip into the renal artery required. This is recognized by screening or by taking appropriate radiographs when the tip takes up a characteristic curve. The accuracy of the position can be checked by a trial injection of a small amount of contrast medium.

The great advantages of these last two methods are that good clear pictures can be obtained with very small quantities of contrast medium, for example, 10 ml. of 35% diodone or 2–3 ml. of 70% diodone. Furthermore, only one kidney is subjected to the possibility of damage and, since all the contrast medium goes into the renal circulation, there is no loss of definition due to filling of arteries not of immediate interest. The disadvantages of these methods are the technical difficulties inherent in the procedures. It is to be hoped that with more experience and improved techniques these difficulties will be overcome.

4. Contra-Indications and Complications

Translumbar Aortography. Impaired renal function is a strong contra-indication to renal angiography by either the translumbar or retrograde femoral method, since the large quantities of contrast medium required for the injection carry a grave risk of accentuating any renal damage which is already present. Should the initial placing of the needle point in the aorta be faulty, it is possible that large quantities of diodone may be injected directly into a major artery, such as the right renal artery or the coeliac axis. It seems almost certain that some of the early fatalities reported were due to superior mesenteric arterial thrombosis arising in this way. In all the fatal cases sodium iodide was used as the contrast medium, and this substance is more irritant than the modern preparations such as diodone. It is unlikely that the severe reactions met when the iodide solution was used will occur with diodone. Nevertheless, unless a trial injection of a small amount of diodone is made in order to determine the exact placing of the needle, the possibility is always present, in this method, of injecting large quantities of contrast medium into a single vessel. Experience has shown that the risk of renal damage is greatly increased where a second injection of 30 ml. of diodone is used. Whiteside (personal communication) states that in no circumstances does he ever repeat the injection if the first injection does not give satisfactory pictures.[1]

Retrograde Femoral Catheterization. As for the translumbar method, large quantities of contrast medium must be injected, but there is no risk of the bulk of the injection passing into any one single artery. Exact placing of the tip of the catheter in relation to the renal arteries is more easily obtained than in the other method. The retrograde femoral method may be quite impossible in patients with tortuous or thrombosed iliac arteries, since in these subjects the catheter may not pass up the aorta.

Selective Renal Angiography. The main difficulties here are technical and, even in the hands of the originators, it is a time-consuming and not invariably successful method. The small quantity of contrast medium required would probably permit its use in cases with impaired renal function, especially as only one kidney is subjected to risk. All retrograde methods carry some slight risk of damage to the femoral artery, with the possibility of subsequent ischaemic changes in the limb.

[1] While this Bulletin was in press, Gayles & Laws (*Brit. med. J.* 1956, **2**, 1141) reported cases of aortic dissection resulting from translumbar aortography.

RENAL ANGIOGRAPHY

(FIGS. A—G)

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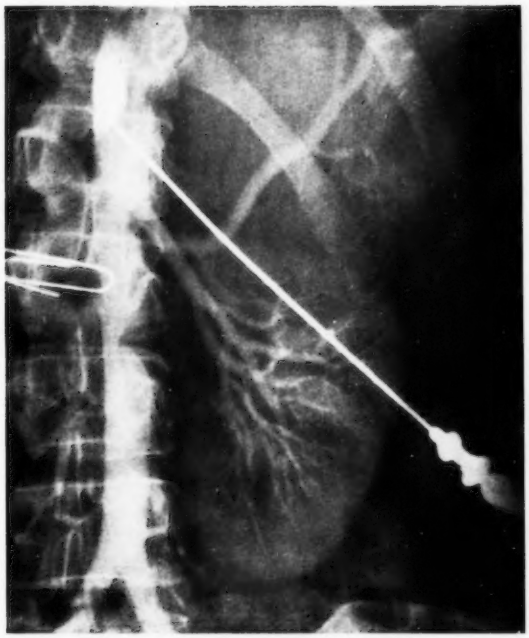

FIG. A. Normal arteriogram of left kidney

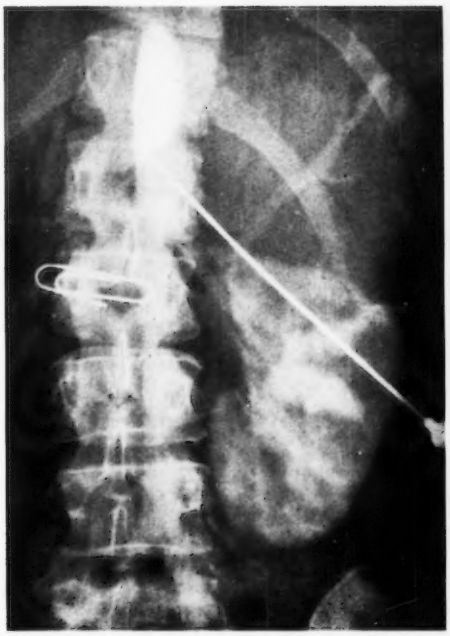

FIG. B. Normal nephrogram of left kidney. Same patient as illustrated in fig. A

FIG. C. Arteriogram showing a large multi-cystic lesion in the lower pole of the right kidney. Some of the intra-renal arteries are displaced and stretched over part of the cyst. No abnormal vessels are present

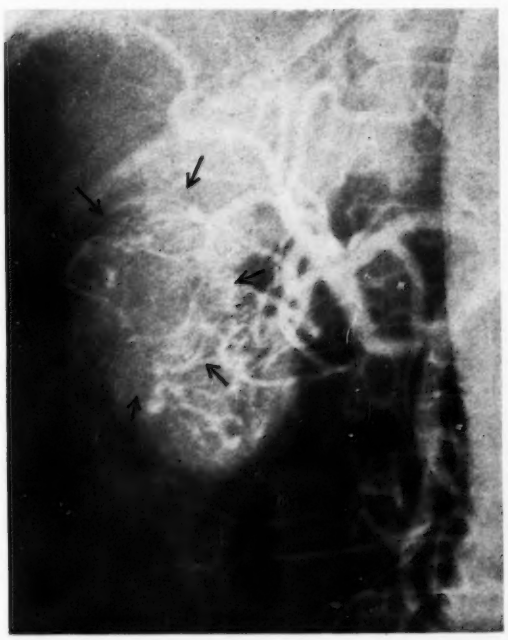

FIG. D. Arteriogram showing typical appearance of a large Grawitz tumour in the right kidney. Tumour vessels are present within the area outlined by arrows

FIG. E. Arteriogram showing early neoplasm in left kidney, outlined by arrows. The site of the tumour is recognized by "pooling" of the contrast medium in the tumour vessels

FIG. F. Intravenous pyelogram showing a peculiar deformity of the right upper calyx. A retrograde pyelogram proved of no assistance

FIG. G. Arteriogram of the case illustrated in fig. F. A collection of large, well-formed, vessels is seen near the upper pole of the right kidney. These are quite different in appearance from the tumour vessels illustrated in fig. D. A diagnosis of angioma was made and confirmed by section of the specimen after nephrectomy. This example illustrates the value of renal angiography in cases with obscure pyelographic appearances

ACKNOWLEDGEMENTS

We are very grateful to Mr E. W. Riches and Dr G. C. Whiteside, who have kindly supplied us with the arteriograms from two of their cases. These appear as figs. D & E (Plates IX & X), by permission of Butterworth, London.

It is probably unwise ever to do angiography by any method in cases of a solitary kidney, or where one kidney only is functioning, since one can never be absolutely certain that no renal damage will ensue. For the same reason it is inadvisable to carry out a nephrectomy immediately after the performance of angiography, the effect of so doing being comparable to performing angiography in a case of solitary kidney.

Paraplegia and hemiplegia have been reported by Evans (1954). It was thought that the spinal theca had been punctured through an intervertebral foramen while the needle was being inserted for translumbar aortography in these cases.

Anuria has been reported by Miller, Wylie & Hinman (1954) in seven cases. These authors suggest that the use of injections at high pressure is the causative factor, but Barr Stirling (1955) has reported a series of cases using a machine employing lever action in order to increase the pressure of the injection beyond that obtainable by manual action, and he had no complications in his series. Eight cases of acute renal failure following aortic injection have been reported by Nils Alwall, Johnsson, Tornberg & Werkö (1955); two of these patients died, and both deaths were attributed by the authors to the effects of the renal angiography.

Neither haemorrhage nor extravasation of the contrast medium is a serious handicap with any of the methods. Idiosyncrasy to the injected material is, of course, to be avoided and most authors feel that preliminary trial intravenous injections should be performed in all cases, if intravenous pyelography has not already been done, using the same contrast medium as will be used for the renal angiography. Should a patient be sensitive to diodone, sodium acetrizoate is a useful alternative contrast medium.

5. Indications

Various authors have shown that renal angiography can be employed in almost any type of kidney lesion, but, after a critical review of the literature and as a result of our own experience, we feel that the investigation is most likely to be of real assistance and to affect significantly the management of the case in a comparatively small proportion of cases. Our views in this respect correspond closely with those expressed by Nesbitt (1955). We feel that at present the main indications for this investigation are:

i. Renal haematuria not explained by any of the simpler urological and radiological investigations. Riches & Whiteside (1956) have recorded at least one example of a very early renal neoplasm, where the diagnosis could only be made by the recognition of an abnormal tumour circulation by translumbar aortography.

ii. In cases of doubtful diagnosis where the pyelographic appearances are obscure or indefinite. This is one of the most frequent indications for the investigation.

iii. Assessment of renal function in cases of hydronephrosis. It is possible to obtain pre-operatively some idea of the amount of functioning renal tissue present. This is done by consideration of the size of the main renal artery and the size and number of intralobar arteries, and by an estimation of the width of renal cortex in the nephrogram picture. Harvard (1953) suggests that renal function can be estimated more accurately by this method than by excretion pyelography. Riches & Whiteside (1956) state that such information supplements that obtained by the surgeon at laparotomy and so helps him to decide whether nephrectomy or a plastic procedure is indicated.

Renal angiography will usually clearly differentiate between simple cysts of the kidney and malignant neoplasms. Although in the majority of cases most surgeons would prefer to explore the lesion (Glaser, 1952), nevertheless there are cases where, for various medical reasons, it is undesirable to subject the patient to a surgical operation if it can be avoided, and for such cases confirmation by angiography that a renal swelling is due to a simple cyst is of considerable help in the management of the case.

Renal angiography may be used to show the size and position of ectopic and/or agenetic kidneys, aberrant renal arteries and other congenital anomalies of the kidneys, but we feel that such indications are very rare and that there is no real justification for using this method of investigation merely to confirm something already known by other simpler methods, or where the surgeon has already decided that exploration should be carried out. Riches & Whiteside (1956) suggest that the knowledge of the state of the renal arteries obtained by angiography is of assistance where partial nephrectomy is contemplated, as in cases of renal calculus, and in some cases of localized tuberculous lesions. We feel, however, that the decision to perform a partial nephrectomy, and the actual amount of renal tissue to be resected, can be made only at operation, after inspection and palpation of the kidney and clamping of appropriate branches of the renal artery. Thus previous renal angiography appears to be only of academic interest in such cases.

Plates IX & X, figs. A–G, illustrate some typical appearances obtained with the translumbar method.

6. Conclusions

We consider that, with careful selection of cases and attention to detail in the performance of the investigation, renal angiography is a worth-while procedure to supplement the simpler diagnostic methods now generally used. In cases where no diagnosis has been made, especially if haematuria has occurred, we feel that renal angiography is indicated. It should never be done merely to confirm something which is already known, or done in cases where the decision to explore surgically has already been made on other grounds. If the method of selective renal angiography can be developed and can be shown to be safe, it is probable that the diagnostic value of the investigation will be increased and the indications for its use widened.

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VENOGRAPHY IN RELATION TO THE KIDNEY

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1 The technique of inferior vena cava and renal vein venography
2 The interpretation of the venogram
a Normal appearances
b Abnormal appearances
References

The inferior vena cava and its tributaries can be satisfactorily outlined radiographically by contrast studies with radio-opaque materials such as diodone. In some types of renal disease associated with venous thrombosis such an investigation can be most helpful in establishing a diagnosis; and also in defining, with some certainty, the site and the extent of a venous block.

Four clinical varieties of renal vein obstruction can be recognized:

i. Inferior vena cava thrombosis with subsequent renal vein thrombosis.
ii. Inferior vena cava obstruction due to direct invasion by a malignant tumour or external compression by malignant disease.
iii. Primary renal vein thrombosis, which is rare except in infancy. Abeshouse (1954) reviewed 228 cases of renal vein thrombosis and found that in 40% the patient's age was under two months.
iv. Renal vein thrombosis secondary to primary renal disease. This may occur in all types of chronic bilateral renal disease, but is especially liable to occur in secondary renal amyloidosis. Vilk (1940) found 13 cases in 249 necropsies of patients with renal amyloidosis. Harrison, Milne & Steiner (1956) reported four cases due to amyloidosis, one due to glomerulonephritis, and one to secondary renal failure in malignant hypertension.

The indications for venography have been defined by Harrison *et al.* (1956). These authors state that venography is of help in the diagnosis of patients with proteinuria, with or without oedema, in the presence of any one of the following abnormalities:

i. Collateral abdominal wall veins with an upward blood flow.
ii. Unexplained oedema of the legs or lower trunk associated with proteinuria of less severity than occurs in the nephrotic syndrome.
iii. Evidence of recurrent pulmonary infarction.
iv. Evidence of malignant disease.
v. A single kidney. Thrombosis of a single renal vein is more likely than simultaneous bilateral renal vein thrombosis.
vi. The nephrotic syndrome occurring for the first time in an elderly patient (in view of the increased incidence of venous thrombosis and malignant disease in this age-group).

Diagnostic accuracy is of great value in assessing the prognosis and deciding treatment, as well as helping to exclude unnecessary surgery for malignant disease. Evidence of vena cava thrombosis is highly suggestive of secondary malignant involvement.

Contra-indications for venography are as follows:

i. A recent thrombo-embolic phenomenon.
ii. Sensitivity to contrast medium, or if the patient is not fit enough to undergo the examination.
iii. Severe uraemia with gross delay of diodone excretion.

1. The Technique of Inferior Vena Cava and Renal Vein Venography

There are two main methods of approach:

i. From below, by the injection of contrast medium through a needle or indwelling polythene catheter into the saphenous vein in the groin, or into the iliac vein.
ii. From above, by catheterization of the inferior vena cava or renal vein via an arm vein through the superior vena cava and right auricle.

The first of these two methods is the simpler and the one indicated in most instances. It will give most of the informaation required about the state of the inferior vena cava and renal veins, provided there is no block of the inferior vena cava. When there is caval thrombosis, however, the inferior route will only outline the lower limit of this block and will demonstrate the collateral circulation. If additional detail is required about the exact site and extent of venous block, particularly the upper end of the caval thrombosis, or when the first method has failed to outline the renal veins adequately, the approach from above through the catheter may have to be resorted to.

Inferior vena cava venography was first described by dos Santos (1935) and since then the method has been used extensively in the investigation of caval thrombosis mainly due to malignant disease (Helander & Lindbom, 1956).

The radiographic technique employed is the same for both methods. The patient is placed supine on a simple cassette tunnel which has been fitted with a stationary grid and can hold 15 × 12 or 17 × 14 in. cassettes. The patient's position must be so arranged that the *x*-ray film will cover the entire abdomen. Before the examination is carried out test films must be taken to check the position of the patient, as well as the radiographic factors. Working at a tube-film distance of 36 in. with a kvp of 94–100 and 70–80 mA, satisfactory radiographs will be obtained. Serial films must be taken to show the complete circulation in the inferior vena cava and its tributaries. Four to five radiographs are exposed at rapid intervals, the first being taken after two-thirds of the contrast medium has been injected, and subsequent films are exposed every two seconds. Films are changed manually when the cassette tunnel is used. As an alternative a mechanical film-changer can be employed, such as the Schonander serial unit. With this machine a larger number of films can be exposed at more frequent intervals and, although this method will give a more detailed picture of the circulation, for practical considerations it is not really superior to manual cassette

changing with the simple cassette tunnel. Once the radiographic requirements are satisfied and the patient has been tested for sensitivity to contrast medium, the examination can be proceeded with. Intravenous injection of 1–2 ml. of contrast medium is an adequate amount to test for sensitivity. An immediate reaction, such as tachycardia, flushing and discomfort, indicates definite sensitivity of the patient to the contrast medium and, unless he has been desensitized, the examination should not be proceeded with (Goodwin, Steiner, Mounsey, MacGregor & Wayne, 1953).

When the first method of injection is used, the patient's skin in the groin is infiltrated with local anaesthetic, and a tributary of the saphenous vein dissected. A polythene catheter of an approximate internal diameter of 2 mm. is then inserted into the vein and slowly advanced for about 2–3 in. into the external iliac vein. During the insertion of the catheter, saline is injected constantly to avoid thrombosis. The catheter's final position must be checked by fluoroscopy and a small amount of contrast medium injected so as to make the catheter radio-opaque. Once the catheter has been adequately positioned, it is connected with the aid of a pressure-screw adaptor to a 100-ml. syringe, and 40–50 ml. of 70% diodone are injected as rapidly as possible, either by hand, or with the aid of a pressure injector (Steiner, 1956). After two-thirds of contrast medium have been injected the first *x*-ray film is exposed and subsequent films are taken at rapid intervals.

To obtain a satisfactory outline of the inferior vena cava with reflux into its main tributaries such as the renal veins, the patient must be instructed to induce a Valsalva manoeuvre at the beginning of the injection, and he must attempt to sustain it throughout the examination, which may last up to 15 seconds. If the patient is unable to sustain the Valsalva manoeuvre for the whole period of the examination, he can relax provided the manoeuvre is satisfactorily induced at the beginning of the injection and then kept up until the second or third film has been exposed. To facilitate the co-operation of the patient the employment of a Valsalva apparatus may be helpful and the patient should be trained in its use before the examination. If the patient is not very co-operative and finds it difficult to carry out the Valsalva manoeuvre during the examination, the procedure should be done under light general anaesthesia. By manual compression of the anaesthetic bag, a very satisfactory Valsalva manoeuvre can then be induced and sustained throughout the examination.

When the second method of injection through a cardiac catheter is chosen, the patient's antecubital fossa is infiltrated with local anaesthetic, and an antecubital vein dissected. A number 9 USA gauge cardiac catheter is then introduced into the vein and under fluoroscopic control threaded along the brachial vein, subclavian vein, superior vena cava, through the right auricle into the inferior vena cava. The catheter tip can then be manoeuvred into the upper end of the inferior vena cava. Brink & Botha (1955) used this position for the demonstration of the upper third of the inferior vena cava and the hepatic veins in the investigation of the Budd-Chiari syndrome. Harrison *et al.* (1956) used this position of the catheter to demonstrate the upper limit of a thrombosed inferior vena cava and were also able to outline the renal vein by retrograde injection of contrast medium. As an alternative the tip of the catheter may be placed in the main renal vein (Steiner, 1956; Dalla Palma & Servello, 1956). During the insertion of the catheter saline is constantly infused to prevent clotting. Once the catheter has been adequately positioned, a syringe filled with contrast medium is attached to the catheter, and with the aid of a mechanical injector contrast medium is injected as rapidly as possible. If the injection is made into the superior vena cava 30–40 ml. of 70% diodone are used; if the injection is made directly into the renal vein only a 50% solution is used and 20–30 ml. are injected. The first film is exposed when half the amount of contrast medium has been injected. Subsequent radiographs are then taken at rapid intervals. The radiographic procedure is similar to that employed with the first method described above.

2. The Interpretation of the Venogram

a. Normal Appearances

When contrast medium is injected into the saphenous vein or external iliac vein, the iliac vein proximal to the site of injection and the inferior vena cava will be clearly outlined. No contrast medium will reflux into the tributaries of the vena cava, nor will collaterals fill. At the point where the renal veins join the vena cava, stream-lining, due to the large inflow of blood constantly diluting the contrast medium, will be noticed (Plate XI, fig. A).

Stream-lining indicates a normal renal-vein flow, and excludes renal vein thrombosis. Absence of stream-lining, however, cannot be interpreted as definite evidence of renal vein thrombosis. When the Valsalva manoeuvre is initiated, temporarily increasing the intraluminal pressure within the vena cava, retrograde flow of contrast medium will outline the renal veins as well as other caval tributaries, such as the hepatic veins, or the opposite iliac veins (Plate XI, fig. B).

When the inferior vena cava is catheterized from above and contrast medium is injected into the renal vein, the main renal vein, as well as its smaller branches, is clearly demonstrated (Plate XI, fig. C). This figure is an example of a retrograde venogram in a patient with the Budd-Chiari syndrome. Contrast medium was injected through a cardiac catheter into the left renal vein. All the main branches of the renal venous system are clearly outlined and one can also see rather tortuous collateral vessels extending from the main renal vein into the lumbar region joining the hemiazygos system, thus by-passing the high caval narrowing. The renal-venous system is perfectly normal.

b. Abnormal Appearances

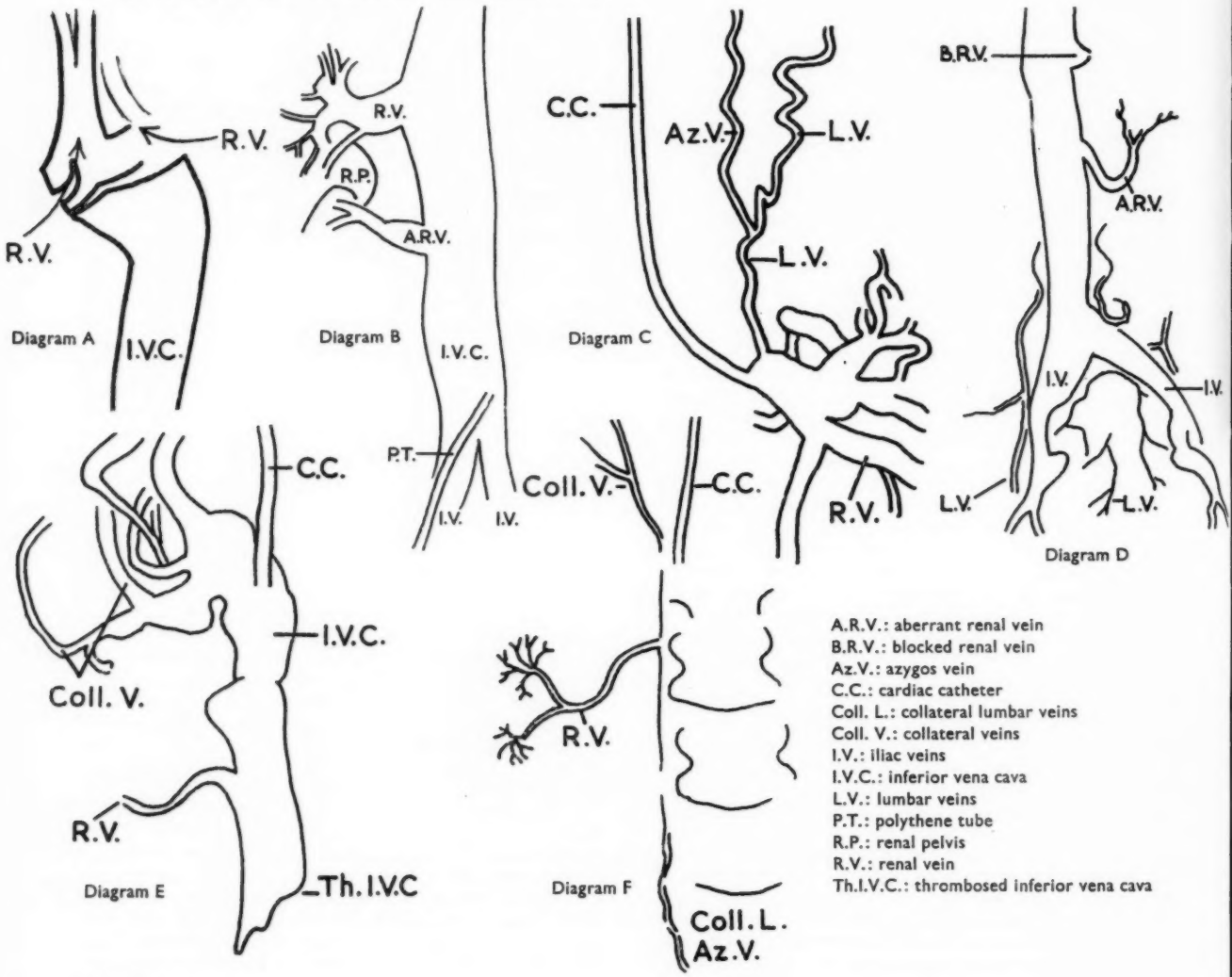
In cases of inferior vena cava obstruction, which may be partial or total, the injection of contrast medium from below via the saphenous or external iliac route will demonstrate the lower limits of the vascular block of the inferior vena cava. It will also demonstrate the extent and type of a collateral circulation through pelvic and lumbar veins, and tributaries of the hemiazygos system. This investigation will not, however, demonstrate the proximal limit of the caval thrombosis; nor will it outline the renal vein satisfactorily.

If the orifices of the renal vein are partially or totally blocked, this will again be clearly shown. Plate XI, fig. D is a venogram of a female patient aged 42 admitted to hospital with a nephrotic syndrome. The venogram carried out through the inferior route demonstrated, after the induction of the Valsalva manoeuvre, thrombosis of the left renal vein and reflux into a small aberrant lower pole vein. Following a fall at the age of 11 resulting in a severe laceration of the right kidney, the patient had a right nephrectomy. She remained perfectly fit up to two months before her present admission to hospital, when she noticed a marked gain in weight, oliguria, and oedema of the limbs, face and back.

When the injection is made from above with the cardiac catheter, the contrast medium will not only outline the proximal limits of a caval block, but also the proximal collateral circulation.

Plate XI, fig. E is a venogram of a female patient aged 50. For three weeks prior to admission she complained of blurring of vision, due to bilateral hypertensive neuroretinopathy with papilloedema. The blood pressure was elevated, 230/130 mm. Hg, and there was proteinuria. In infancy this patient had a left nephrectomy for a renal carbuncle, and since the age of 14 dilated abdominal wall veins were present—collateral vessels, presumably the result of an inferior vena cava thrombosis following on the nephrectomy. The venogram was carried out by the upper route, the catheter being placed in the proximal third of the inferior vena cava, and contrast medium injected at that level. It shows complete caval thrombosis in the lower third with partial thrombosis in the upper third. With the aid of the Valsalva manoeuvre, reflux of contrast medium into the renal vein on the right side was achieved. The second serial film taken within two seconds (Plate XI, fig. F) shows a grossly narrowed renal vein, with some abnormal smaller branches which are partly blocked.

In the experience of Harrison *et al.* (1956), the methods of venography which they used in their investigation of the clinical aspects of renal vein thrombosis proved perfectly harmless and there were no complications. Dalla Palma & Servello (1956) confirmed their conclusions.



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VENOGRAPHY IN RELATION TO THE KIDNEY

(FIGS. A—F)

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FIG. A. Normal venogram of the inferior vena cava, showing stream-lining due to flow of blood from renal veins

FIG. B. Venogram of inferior vena cava with the Valsalva manoeuvre

FIG. C. Retrograde venogram through a cardiac catheter

FIG. D. Venogram by the inferior route, which demonstrates thrombosis of a main left renal vein, the partial filling of an aberrant inferior pole vein, and some collaterals in the pelvis

FIG. E. Venogram of the vena cava by the upper route, which demonstrates a thrombosed lower third of the inferior vena cava and a very small right renal vein with some collaterals in the region of the hepatic vein

FIG. F. Same as fig. E. Film exposed 2 seconds later

NOTE. The diagrams on p. 66 are intended for comparison with these figures.

PLATE XII (FIGS. A—D)

EFFECTS OF OLD AGE AND OVER-NUTRITION ON THE KIDNEY

(FIGS. A—D)

G. C. Kennedy

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FIG. A

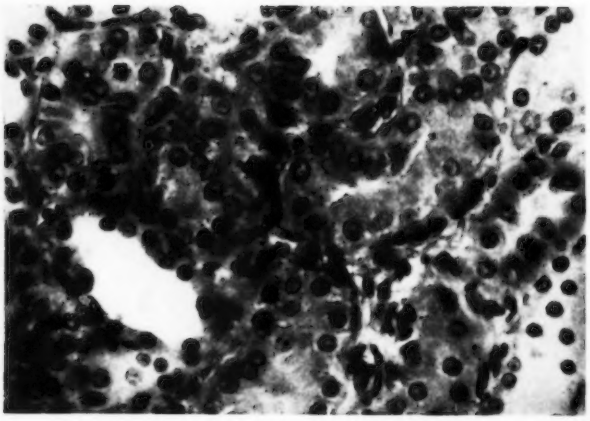


FIG. A. Proximal convoluted tubules from a "normal" rat 21 months of age. The epithelium in the top and left part of the field is hyperplastic, while the lower right part contains normal tubules. Note the dark staining of the cytoplasm of the dividing cells

FIG. B. Collecting tubules from the kidney of the 21-month-old rat. Note the abnormal cellularity and the presence of casts in the tubules

FIG. B

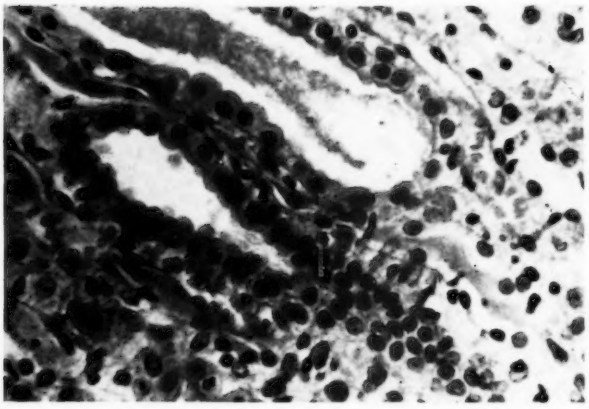


FIG. C

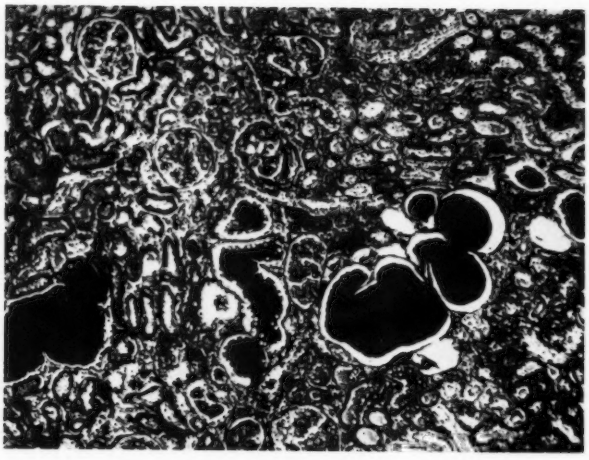
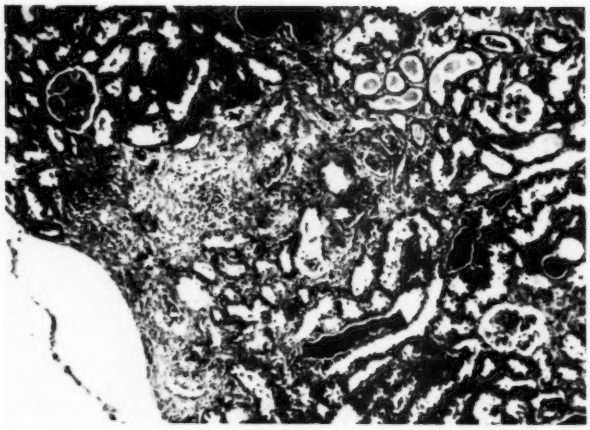


FIG. C. Low-power view of advanced renal lesions from a senile rat (27 months of age), showing dilated and atrophic tubules and glomerular lesions

FIG. D. Radial scar in kidney of 27-month-old rat

FIG. D



EFFECTS OF OLD AGE AND OVER-NUTRITION ON THE KIDNEY

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1 The senile kidney of the rat
a Age of onset of lesions
b The lesions: development and final form
2 Production of hyperplasia in the kidney
3 Late effects of hyperplasia in the kidney
4 Summary and conclusions
References

We are all aware that as time passes we deteriorate physically and even in the absence of disease must eventually die. Senescence is not a universal characteristic of living material, but has been acquired during evolution. Minot (1908) first suggested that mammalian ageing was related to the loss of growth and regenerative capacity in differentiated cells, and paradoxically was most rapid when growth was most rapid. An interesting corollary of this view is that individual tissues may age at rates which vary with their degree of differentiation, and not synchronously with the body as a whole. The transient tissues associated with reproductive function are the most extreme example of this. Apart from its obvious developmental association with the gonads, the metanephros has many of the characteristics of such a transient tissue. It is the third organ of essentially similar structure to arise during development. It grows rapidly, so that in most mammals it is structurally fully developed at birth or soon afterwards, and in postnatal life it is incapable of forming nephrons. In the rat, which has been most extensively studied, it has been shown that there is progressive disappearance of nephrons after middle life (Arataki, 1926).

In old age the kidneys of many mammals, including man, differ histologically from the normal kidney of youth, but it is not always easy to distinguish the end-results of senile atrophy from those of pathological injury. Oliver (1942) sectioned the kidneys of 75 persons over the age of 70 who had shown no signs of renal disease during life. In every case he found degenerative and atrophic changes, but these were always accompanied by arteriosclerosis. He suggested that renal ageing was primarily an ischaemic process. It is clear, however, that he regarded arteriosclerosis as itself a physiological accompaniment of old age, and he even extended his ischaemic theory to explain the decline of the mesonephros, shown by Gersh (1937) to be accompanied by a diversion of blood supply to other structures.

Actuaries have shown that obesity represents a major hazard of middle life. The premature death of fat men and women is not due as a rule to any new or specific disease, but to the acceleration of the onset of causes of death common in people of normal weight, particularly of cardiovascular and renal disease (Preble, 1923; Dublin, 1930; Dublin & Marks, 1939). Obesity in rats can readily be produced in a severe form by obliteration of centres in the hypothalamus which control the intake of food; rats treated in this way will double their intake for a period of six weeks to three months. This provides a means of studying the problem experimentally, and Brobeck, Tepperman & Long (1943) have shown that rats treated in this way often developed "chronic glomerulonephritis". This has been confirmed (Kennedy, 1951, 1955), but the lesions have been shown to be identical with those that occur spontaneously in senility. The present article gives a preliminary account of investigations, as yet unpublished, of some of the chemical changes in the kidneys of obese or ageing rats, which amplify the previous histological studies.

1. The Senile Kidney of the Rat

a. Age of Onset of Lesions

McCay (1942) estimated that the average life-span of the rat in captivity was about 600 days, and this agrees well with our experience. In the present study, groups of five rats were killed at intervals of three months, and the kidneys of those that died spontaneously were also examined; no renal abnormalities were found before 21 months. The commonest cause of accidental or natural death before that period was pulmonary infection, while natural death over the age of two years was very frequently associated with severe renal damage. Renal damage of this degree developed in obese rats between a year and 15 months of age (Kennedy, 1951).

b. The Lesions: Development and Final Form

The first histological abnormality detected in our rats was an increase in the number of epithelial cells of occasional tubules, usually in the proximal convolutions. The new cells were smaller and more basophilic than normal, often arranged in more than one layer without the usual brush border (Plate XII, fig. A). Mitotic figures, which are very rare in normal tubular cells, were common. The parietal epithelium of the capsule standing at the head of the affected tubule was frequently also hyperplastic. In other nephrons the hyperplasia was more extensive, and rapidly dividing cells were seen in the collecting tubules, which in some cases were so obstructed as to give the appearance of solid columns of cells. Above these obstructions, protein casts could be seen (Plate XII, fig. B). The kidney at this stage was moderately enlarged but macroscopically normal.

As age increased, kidneys were often found to be greatly enlarged and granular in appearance to the naked eye and to show wide-spread tubular dilatation and atrophy, together with glomerular lesions similar to those of chronic glomerulonephritis. Many glomeruli were fibrosed, while in others the capillary tuft was bloodless and adherent to Bowman's capsule (Plate XII, fig. C). The glomerular basement membrane was usually thickened. Only in the final stage was any vascular narrowing apparent, associated with wedge-shaped scars reminiscent of the human arteriosclerotic kidney (Plate XII, fig. D). Reliable blood-pressure readings were not obtained by the tail plethysmograph method because of thickening and ulceration of the tail skin, but the heart was found to be grossly enlarged when the renal lesions were advanced. In many rats, however, even with fairly severe renal damage, the renal vessels and heart size were quite normal, and it seemed unlikely that the renal damage was primarily due to a reduction in the blood supply. There was a variable amount of round-cell infiltration

of the interstitial tissue round the atrophic dilated tubules, but nothing suggesting either a focal or an ascending urinary infection. These changes were accompanied by the excretion of urine containing large amounts of protein and numerous casts.

These findings are in general agreement with those of previous investigators. Authors have differed, however, in their interpretation of the pathological appearances. Wilens & Sproul (1938) were primarily interested in the incidence of spontaneous cardiovascular disease, and, when they found little renal arteriosclerosis, concluded that the lesions were those of chronic glomerulonephritis. Saxton & Kimball (1941) were impressed by the heavy albuminuria and dilated tubules. They classified the changes as those of nephrosis. Goldblatt (1947) referred to the "chronic pyelonephritis" which he encountered in his colony. It should be emphasized, however, that these different diagnoses were all based upon comparison of the histological appearance of terminal lesions in dead or moribund rats with the more common diseases of the human kidney, rather than upon a consideration of the life story of the rat's kidney.

Similar histological changes have been observed in ageing mice by a number of workers. The final stages, as in rats, resembled those of chronic or healed pyelonephritis of man, as described by Weiss & Parker (1939). Dunn (1944) and Thung (1955) regarded the primary lesion as occlusion of the collecting tubules by amyloid deposits. Twort & Twort (1932) reported similar changes in mice infested with *Klossiella muris*, but neither of the later workers quoted found any evidence of this in their mice. There was no amyloid infiltration of the kidneys of our rats.

However one chooses to describe the terminal lesion in rats, the real destroying agent appears to be misdirected hyperplasia of parts of the tubular epithelium. Rapid destruction of the kidney of normal young rats by the excessive hypertrophy induced by the combination of unilateral nephrectomy and a high-protein diet was demonstrated by Addis and Oliver (Oliver, 1945). Chanutin & Ferris (1932) showed that a more extensive partial nephrectomy, in which one kidney and two-thirds of the other were removed, resulted in renal lesions even though the diet contained only normal amounts of protein. These experiments have now been repeated, and extended to a study of the effect of the age of the rats at the time of the operation. The changes occurring in the overloaded kidney have been compared, both histologically and chemically, with those that were found in old age and in obesity. It has been concluded that excessive regenerative growth, old age, and obesity are all characterized by the production in the renal tubules of new cells in such numbers that obstructive lesions occur and a vicious cycle is set up in which more and more tubules become functionless. The protein load that is necessary to produce such changes in the kidney diminishes with age, and the reason for this is discussed later in this article.

2. Production of Hyperplasia in the Kidney

Visceral growth and the stimuli which produce it are not yet fully understood. It is sometimes assumed that the regeneration that follows partial removal of a viscus is identical in nature with the growth that follows an increased functional demand upon the organ. Verzár (1955) writes ". . . compensatory hypertrophy in consequence of increased function can be seen after extirpation of one kidney or one adrenal, or in the liver after extirpation of one lobe". Much recent work has shown, however, that the histological and chemical changes during these two types of growth may be quite different. This is particularly clear in the liver, in which regeneration is characterized by an almost immediate onset of cell division throughout the organ, with a high rate of mitosis within 48 hours (Higgins & Anderson, 1931; Brues, Drury & Brues, 1936; Price & Laird, 1950). The growth that follows the consumption of a high-protein diet, which is the method usually employed to increase functional demand, consists of an increase in the size, but not in the number, of liver cells (Campbell & Kosterlitz, 1950; Davidson, 1953). In the kidney, regeneration is accompanied by less rapid production of new cells than in the liver. Rollason (1949) showed significant mitosis histologically on the second day after unilateral nephrectomy in young rats, but Mandel, Mandel & Jacob (1950) demonstrated chemically that the principal increase in deoxyribonucleic acid (DNA) in regenerating kidney in adults occurs between three and six weeks after operation. Deoxyribonucleic acid is confined to cell nuclei and is present in constant amount in the nuclei of a given species, and so provides a convenient chemical way of finding the number of cells in a tissue. It will be shown that doubling the food intake has structural effects upon the kidney similar to those of unilateral nephrectomy, so that one kidney can be said to react to x grams of food in much the same way as two kidneys to 2x grams. In stressing this similarity of effect in the following discussion, however, it is not intended to imply that the cause is necessarily the same.

The hyperphagia which follows hypothalamic operation is at a maximum immediately, and in the first week the daily intake of food may exceed a quarter of the body-weight. The gastrointestinal tract, liver, kidney and heart grow rapidly. The maximum visceral weight is achieved more quickly than the maximum obesity and, while the first is an increase in protein, the second is almost entirely fat. The liver may double in weight within two weeks, and the kidney rather more slowly, while a corresponding increase in body-weight takes about two months. For this reason, and since a similar increase in visceral size is found in lactation in which there is a high food intake but no obesity, it has been concluded that the visceral growth is a reaction to high food intake rather than to obesity (Kennedy, 1955).

Table I shows results of some recent unpublished experiments. The chemical changes found in the kidney during hypothalamic hyperphagia are compared with those during

TABLE I. COMPOSITION OF THE KIDNEYS OF OBESE RATS, OR OF RATS WITH ONE KIDNEY REMOVED, AT THREE AND SIX WEEKS AFTER OPERATION

Time from operation	Group	Total nitrogen (mg. per kidney)	Ribonucleic acid phosphorus (mg. per kidney)	Deoxyribonucleic acid phosphorus (mg. per kidney)
	Control (not operated on)	19.4	0.378	0.183
3 weeks	Kidney removed	27.8	0.504	0.210
3 weeks	Obese	29.9	0.488	0.227
6 weeks	Kidney removed	29.4	0.516	0.289
6 weeks	Obese	28.3	0.532	0.308

regeneration after unilateral nephrectomy. The first line shows the nitrogen and ribonucleic acid (RNA) content of an average normal kidney and its DNA content. Nitrogen and RNA are indices of the amount of cytoplasm present, and DNA of the amount of nuclear material, and therefore of the number of cells. It will be seen from the next two lines that the increase in nitrogen and RNA three weeks after hypothalamic operation or unilateral nephrectomy was substantial and much the same in both cases, while the change in DNA was slight. At six weeks after operation there was little further increase in the cytoplasmic constituents, but the DNA had risen in proportion with them, indicating that the number of cells had increased, with a restoration of the average cell size to normal. At this stage histological examination revealed a large but structurally quite normal kidney. Since the immediate effects of the stimuli were so similar, the later effects upon the kidney seemed to be worth further study.

3. Late Effects of Hyperplasia in the Kidney

It has already been noted that renal lesions histologically identical with those found in the " normal " rat two years of age appeared in obese rats about nine months earlier—between one year and 18 months of age. Ten rats had one kidney removed at three months of age, at the same period as the hypothalamic operations were usually performed. Five were killed at 15 months of age; the kidneys of two showed destructive lesions of old age, while nephrons in the other three were enlarged and hyperplastic. The remainder of the rats were killed at 18 months of age and all their kidneys were highly abnormal. The extent of the hyperplasia which occurs may be judged from Table II, in which the same chemical

TABLE II. COMPOSITION OF THE KIDNEYS OF OBESE ANIMALS, OR OF ANIMALS WITH ONE KIDNEY REMOVED, TWELVE MONTHS AFTER OPERATION (OPERATIONS AT THREE MONTHS OF AGE)

Group	Total nitrogen (mg. per kidney)	Ribonucleic acid phosphorus (mg. per kidney)	Deoxyribonucleic acid phosphorus (mg. per kidney)
Control (not operated on) . . .	34.9	0.749	0.239
Kidney removed. .	73.5	1.429	0.695
Obese . . .	75.9	1.972	0.785

parameters as in Table I are compared. The control values are higher than in Table I, reflecting the difference in age of the animals. The increase in DNA in the abnormal kidneys is much greater than in Table I, however, and has kept pace with the increase in RNA and nitrogen.

Since both the immediate and the delayed effects of overfeeding and regenerative hyperplasia were so similar, it seemed likely that the characteristic changes in the kidney might be accelerated by combining the two stimuli. Three-month-old rats were accordingly unilaterally nephrectomized and made obese. The latent period before kidney lesions appeared was found to be shortened from twelve months to six.

It next seemed important to find whether the latent period was dependent upon the age of the animal. Hypothalamic puncture or unilateral nephrectomy was, therefore, performed on groups of rats at six months and at one year. It was found that kidney damage developed more quickly with increasing age of the animals. At the same time, however, this may not have been due to specific ageing changes in the cells of the kidney, but to the fact already mentioned that from 300 days of age onwards there is a progressive loss of nephrons from the rat kidney (Arataki, 1926). This process, which has been shown by Moore & Hellman (1930) to go on even in hypertrophied kidneys, is equivalent in its effect on the surviving nephrons to the surgical removal of further renal tissue.

Brobeck (1946) and Long (personal communication, 1956) suggested that the kidney damage in obese rats might be due to disproportionately high choline requirements. Hartroft & Best (1949) showed that chronic kidney lesions similar to those of old age were found in choline-deficient rats which survived for a few months after the infantile " haemorrhagic " stage. However, it appeared doubtful whether the chronic lesions were directly due to choline deficiency, since they occurred only in rats in which the acute damage had been very severe. Hartroft and Best pointed out the analogy with the experiments of Chanutin & Ferris (1932) in which the maximum surgical removal of kidney tissue compatible with immediate survival had a similar effect on the kidney. After such extensive operations, three-month-old rats of our colony survived for about six months, about the same time as after the combination of unilateral nephrectomy and hypothalamic operation.

It will be seen that in all these experiments the effect of simple over-nutrition on the kidney both immediately and in the long term is equivalent to that of the removal of one kidney from a normally nourished animal. It is unnecessary, therefore, to postulate any damaging effect on the kidney or its vessels from adiposity.

4. Summary and Conclusions

Over-feeding increases first the size and then the number of cells in the kidney. A similar effect follows unilateral nephrectomy. After a latent period of some months each stimulus leads to the premature appearance of a type of kidney lesion common in senile rats. The latent period can be shortened by combining the stimuli, by more extensive removal of renal tissue, or by using older rats. It is suggested that the gradual loss of renal substance which is characteristic of ageing is analagous to partial nephrectomy, in its effect on the surviving tissue.

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PROFESSOR CLIFFORD WILSON is Professor of Medicine in the University of London and Director of the Medical Unit at The London Hospital. He has previously contributed an article to the Bulletin (*Brit. med. Bull.* 1952, **8**, 316) on experimental hypertension, with which a biographical note was published.

DR OLIVER WRONG is Tutor in Medicine in the University of Manchester. After qualifying in Oxford and serving in the Royal Army Medical Corps, he travelled in Canada and the USA on a George Herbert Hunt Travelling Scholarship, holding appointments at the Toronto General Hospital and at the Presbyterian Hospital, Chicago. Subsequently he worked with Dr Alexander Leaf at the Massachusetts General Hospital, Boston. He has published work with Dr Leaf and others on the distribution of body-water and the excretion of water and electrolytes, and is at present engaged on the clinical study of renal tubular function.

Book Reviews

Modern Views on the Secretion of Urine

Edited by *F. R. Winton*. London: J. & A. Churchill Ltd., 1956. (The Cushny Memorial Lectures.) vii + 292 pages; figures. 22 × 15 cm. £1 10s.

This book contains a series of ten lectures on the kidney, given for the most part by past and present members of the staff of University College, London. The intention was that they should have been given entirely by members of the College, but it was found that not every subdivision of kidney function was covered by its experts. How surprised Cushny, whose interest spanned the field of medicine, would have been to discover that even ten renal specialists can scarcely do justice to the complications of renal function.

Cushny had no difficulty in explaining how the kidney worked—there was filtration and reabsorption from the filtrate of an "Ideal Locke's Solution". S. E. Dicker, in the first chapter of the book under review, holds that filtration does go on, but expresses doubt as to the accuracy of its measurement. It is suggested that inulin clearance as a measure of glomerular filtration rate should be " . . . considered as a working hypothesis rather than as a revealed truth". In the second chapter, by Grace Eggleton, we would appear to have left the "Ideal Locke's Solution" far behind: for some substances filtration, reabsorption and secretion can all be going on at the same time. F. R. Winton has a new and interesting approach to the problem of how the kidney manages to keep its blood flow relatively constant in the face of movement of arterial blood pressures. It is suggested that the blood entering the kidney is split into two streams, one plasma-rich and the other cell-rich. Control of renal blood flow by such a process could be very fine indeed, and although evidence is given for the presence in the kidney of this "dynamic haematocrit", we are not told how it is effected.

L. E. Bayliss examines the process of secretion and, although this is the process Cushny rejected as being vitalistic, alleging that we could never understand it, the text would seem to show he was unduly pessimistic. In the chapter on the genetic aspect of tubular function, H. Harris establishes a heredity basis for a number of renal disorders, which are of importance because they each present a ready-made experiment on the kidney.

There is a chapter by Mary Pickford on antidiuresis, and one by J. D. N. Nabarro on the adrenal cortex and renal function. R. A. McCance & E. M. Widdowson write on renal function in the first two days of life, M. L. Rosenheim on functional aspects of renal failure, and G. M. Bull on osmotic diuresis in acute tubular necrosis.

This book presents an account of the present position in renal physiology, with which probably the majority of workers in this field would agree. It has the merit that the chapters make no assumption of special knowledge on the part of the reader—anyone with even an elementary knowledge of the terms used in renal physiology should be able to follow the arguments.

J. A. Barclay

Practical Urology: Case-Comments and Late Results

Alex E. Roche. xii + 258 pages; 132 illustrations. 22 × 14 cm. London: H. K. Lewis & Co. Ltd., 1956. £1 15s.

The abbreviated title of this little volume suggests a practical guide to routine urological investigation and procedure. Nothing so pedestrian, however, is in the author's mind when he sets out to record and analyse what are described as a " . . . haphazard array of cases, collected during many years", which are now selected either for their interest or on their instructional merits.

Urology is a subject in which the blending of clinical and radiological information often provides such exceptional opportunity for reliable diagnosis as to give scope for subsequent refinements in practical management. The choice of appropriate procedures, however, depends largely on experience, and it is the author's aim to inculcate the lessons learned from a careful study and follow-up of his personal cases. The latter are arranged in chronological order as they were encountered during the past 25 years, and they include an assortment of commonplace conditions rendered interesting by special circumstances, interspersed with others of greater rarity or grotesqueness. Each is described with factual and facetious comment combining to leave a vivid impression of the thought expended in overcoming individual problems. The book runs on through 51 cases of urological interest, commencing with a strange example of stones in a urethral pouch and terminating with a description of "Cure of dyspareunia etc. by L. nephrectomy for ectopic kidney!" Many useful points of practical importance emerge in the intervening sections, particularly in relation to conservative surgery and to the avoidance of various snares and pitfalls in urological diagnosis and treatment. To those interested in the specialty there should be no difficulty in construing the abbreviations with which the text abounds, though these may prove irksome to the fastidious reader. The wealth of first-hand experience on which the author draws more than compensates for minor inconveniences of this nature, and the book can be warmly commended, both on its practical merits and as an interesting interlude, to all those interested in urology.

J. D. Fergusson

Body Fluids in Surgery

A. W. Wilkinson. Edinburgh & London: E. & S. Livingstone Ltd., 1955. ix + 212 pages; 7 figures + 29 tables. 22 × 15 cm. 16s.

The management of disorders of water and electrolyte metabolism is a complex subject which frequently requires a working knowledge of both renal and cellular physiology. It is perhaps this aspect which has tended to deter surgeons from taking a detailed interest in the subject, although these disorders occur most commonly in surgical cases.

However, the manifest importance of water and electrolyte imbalance has given rise to several monographs on the subject, and the present volume, written for surgeons by a surgeon, is a most welcome addition. The author has set out the cardinal principles of his elaborate subject as simply as possible, with the result that the book makes easy reading. However, at the same time, sufficient detail is included, with the necessary theoretical background, to provide a very comprehensive account.

The book is most up to date, with descriptions of recent important work, such as that on intracellular acidosis associated with certain forms of potassium depletion, and on the ion-exchange mechanisms across the renal tubule cell. Newer forms of treatment, such as the proposed use of intravenous ammonium chloride solutions in alkalosis, have been critically assessed.

Perhaps a little too much emphasis is placed on potassium deficiency as a frequent cause of alkalosis in surgical cases. There are certain inaccuracies, especially when the author discusses renal function. Thus it is hardly correct to say that glomerular filtration rate depends on renal blood flow, or that urea is not absorbed by the renal tubules.

However, these inaccuracies could easily be corrected, and scarcely detract from the value of the book as a whole. It is hoped that as many surgeons as possible will read this short and valuable work.

J. R. K. Preedy

Refresher Course for General Practitioners: Specially Commissioned Articles from the British Medical Journal

British Medical Journal. Third collection April 1952 to September 1953 (fully revised). London: British Medical Association, 1956. xvii + 548 pages; illustrations. 22 × 15 cm. £1 5s.

This is the third collection of the well-established refresher course articles for general practitioners appearing each week in the *British Medical Journal*. The period under review is from April 1952 to September 1953, but each article has been revised to bring it up to date. The topics are varied, ranging from antenatal care to certification of death. The general practitioner requires a good working knowledge of all the subjects dealt with in this book, and it serves its purpose well by reminding him of what is old and well established, and informing him of new developments. Each contributor is an authority on his subject; Robert Platt, for example, writes the article on nephritis. This is a useful book, but it is unlikely to appeal to the specialist.

I. Crawford Jones

Scientific and Learned Societies of Great Britain: a Handbook Compiled from Official Sources

58th ed. London: George Allen & Unwin Ltd., 1956. 211 pages. 22 × 14 cm. £1 15s.

The second post-war edition of this book, which is now edited by The British Council, lists by subject over 600 scientific and learned societies of Great Britain. In part I are given names and addresses of the main research establishments administered by the Department of Scientific and Industrial Research, the Medical Research Council, and the Agricultural Research Council. There is also a select bibliography of relevant publications. Part II is divided into 16 sections, including medicine, biology and microscopy, chemistry, and general science. There is a new section on university societies. Each entry in this part gives as many details as possible of the objects, membership, publications, library and so on, of the society. Anyone wishing to obtain information of this kind will find this a useful reference book.

O. M. H.

Blackwell Scientific Publications: Complete Catalogue, 1957; Medicine and Science

Oxford [1956]. Blackwell Scientific Publications Ltd. 138 + 2 pages. 22 × 15 cm. (Free on application to the publishers)

This catalogue contains over 720 titles of medical textbooks and monographs, and is divided into 72 sections under headings ranging from alcoholism to zoology. Each entry gives full bibliographical details of the publication, with, in some cases, comments or notes on its contents. The books of Blackwell Scientific Publications Ltd. and those of Charles C. Thomas, Springfield, Illinois, are published simultaneously in the USA and Great Britain, and in Canada by the Ryerson Press, Toronto. The catalogue may be obtained on request from the publishers, 24–25 Broad Street, Oxford, England.

O. M. H.

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CHEMISTRY OF THE LIVER CELL

(FIGS. A, B)

J. N. Davidson

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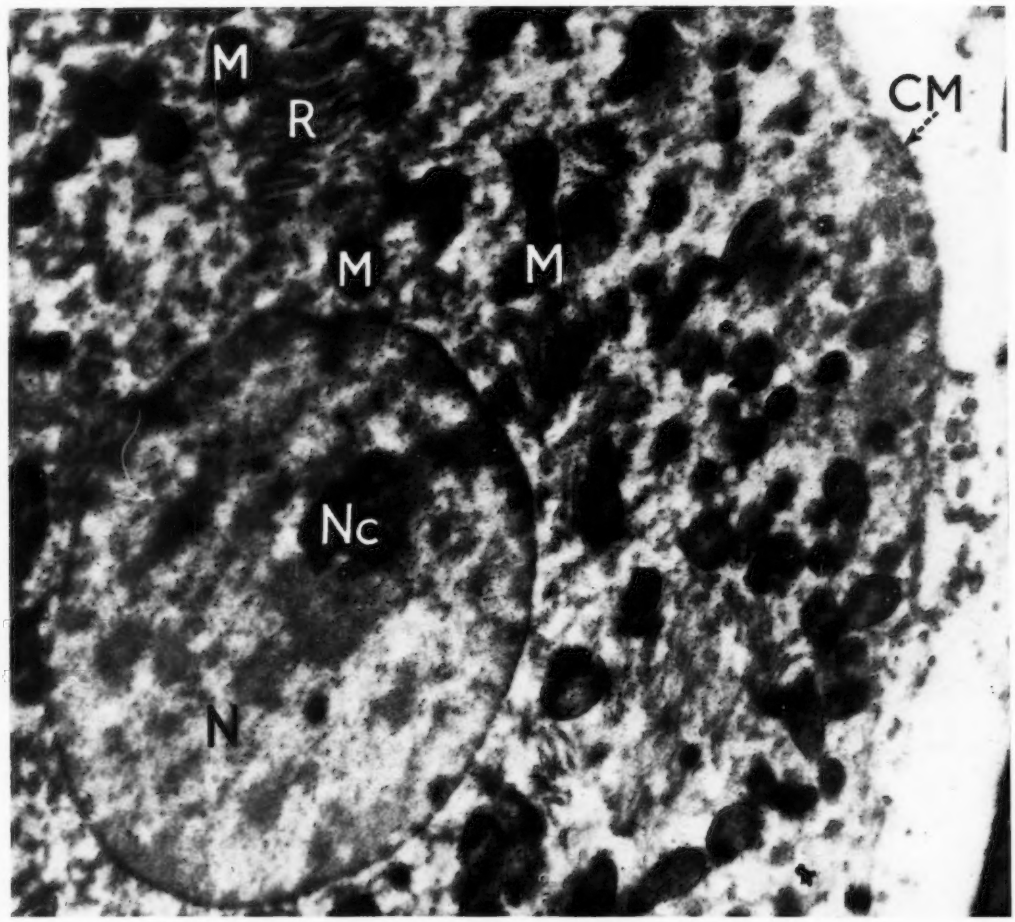

FIG. A. Electron micrograph showing section of part of a liver cell
N: nucleus; Nc: nucleolus; M: mitochondria; R: endoplasmic reticulum; CM: cell membrane
(magnification × 10,000)

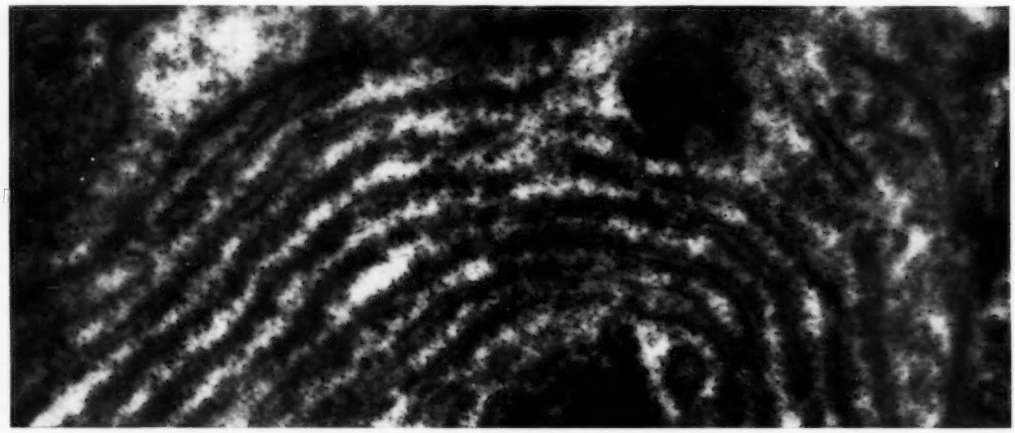

FIG. B. Electron micrograph showing part of the endoplasmic reticulum at higher magnification. The dense particles on the outer surface of the limiting membrane are clearly visible
(magnification × 40,000)

(By courtesy of Professor G. M. Wyburn and Dr I. M. Dawson)

CHEMISTRY OF THE LIVER CELL

J. N. DAVIDSON M.D. D.Sc.

Gardiner Professor of Physiological Chemistry
University of Glasgow

1 The fine structure of the liver cell
2 The separation of cell components
3 Properties of the subcellular fractions
4 Composition of liver tissue
5 Influence of dietary changes
6 Conclusion
References

So much work has recently been carried out on the chemical composition of the subcellular components of the liver cell that it is appropriate to begin this paper with a brief survey of the morphological aspects of the problem. The minute structure of the liver lobule has recently been reviewed by Hale (1951) and need not be further considered. It is, however, important to bear in mind that the liver is far from homogeneous in cell content, since it contains parenchymatous cells or true hepatocytes, Kupffer's cells or macrophages, epithelial cells of bile ducts, etc., and fibroblasts. Although the most abundant of these, the hepatocytes, comprise only 60% of the total cells in the liver, it is on these that most work has been concentrated.

1. The Fine Structure of the Liver Cell

During the last few years the fine structure of the liver cell has been intensively studied in the electron microscope, especially in the Rockefeller Institute in New York, and in Sjöstrand's laboratory in Stockholm. The main features are illustrated in the electron micrograph reproduced in Plate I, fig. A, which shows the nucleus containing a mesh-work of material referred to by the histologist as chromatin and by the biochemist as deoxyribonucleoprotein. In this mesh-work one or more nucleoli are embedded. The cytoplasm contains numerous mitochondria (of dimensions 0.5–5 μ. × 0.3–0.7 μ.), the fine structure of which has recently been discussed by Palade (1953, 1956). Each mitochondrion is bounded by an outer and an inner membrane about 5 mμ. in thickness, the inner membrane being connected with a series of incomplete partitions, the cristae mitochondriales, which project into the interior of the organelle, dividing it into a series of interconnecting compartments.

The liver cell also contains granules of glycogen, lipid inclusions and secretory granules as well as such easily recognized features as the Golgi apparatus. But probably the most interesting cytoplasmic component as revealed by electron microscopy is a complex mixture of strands and vesicles which Porter (1953) has termed the " endoplasmic reticulum " (Plate I, fig. B). It is limited by a membrane about 5 mμ. thick separating the content of the tubules and vesicles from the general matrix of the cytoplasm and giving the whole component the character of a finely divided vacuolar system, which has been identified with the basophilic or chromophilic component (the ergastoplasm) of the cytoplasm.

The structure of the endoplasmic reticulum in the liver cell has been intensively studied in very thin sections in the electron microscope by Palade & Siekevitz (1956) who found that it could be represented by numerous profiles of circular, oval or elongated shape with smaller diameter measuring 40–150 mμ. They are bounded by a very fine membrane and have an apparently homogeneous content. Two types of profile can be distinguished, smooth and rough surfaced. The smooth-surfaced profiles (40–100 mμ. in diameter) are circular, oval or irregular in shape and correspond to vesicles and contorted tubules linked together in a tightly-meshed reticulum. The rough-surfaced profiles are more numerous and are of length 50 mμ. to 5 μ., with a fairly constant diameter of about 50 mμ. They frequently occur in more or less parallel arrays separated from each other at fairly regular intervals (Plate I, fig. B). The rough surface is due to the presence of small dense particles (10–15 mμ. diameter) attached to the outside surface of the limiting membrane. These particles, which are absent from the smooth-surfaced profiles, are exceedingly important on account of their very high content of ribonucleic acid (RNA). As we shall see later, they form an essential part of the microsomes.

2. The Separation of Cell Components

While the composition of these cellular constituents may be studied in situ with the aid of some of the elegant biophysical techniques developed, among others for example, by Caspersson (1950, 1955) in Stockholm and by Randall and his colleagues in London (Davies, Wilkins, Chayen & La Cour, 1954; Walker, 1955; Deeley, 1955), from the chemical point of view most information has been obtained by the study of cell components separated by the process of differential centrifugation from cells disrupted in bulk in a suitable medium.

The original classical experiments on the separation of subcellular fractions were carried out by Bensley and by Claude (see for example Claude, 1943) and resulted in the separation of liver tissue disrupted in saline solution into a nuclear fraction, a fraction of large granules including mitochondria, a fraction of small granules including microsomes, and a supernatant fraction free of sedimentable material. Fractions consisting of particles smaller than the microsomes have been obtained by Barnum & Huseby (1948) who called them " ultramicrosomes " and by Petermann, Mizen & Hamilton (1953) who used the term " macromolecules of ribonucleoprotein ". Much of the recent work in this field has been carried out by Hogeboom & Schneider who have reviewed the subject in detail (1955) and whose procedures are now generally followed.

The fractionation of cells in saline solution is complicated by a tendency for the granules to aggregate and to sediment as clumps rather than as discrete particles. This tendency can be overcome by using 0.88 M-sucrose as a dispersion medium in which the mitochondria retain their rod-like morphological characteristics and their ability to stain supravitally with the dye Janus green B. At this concentration of sucrose, however, on account of the high viscosity and density of the medium, very high centrifugal speeds have to be employed to sediment the subcellular fractions, and the modern practice is to use 0.25 M-sucrose as a medium in which aggregation of granules does not occur and in which

mitochondria can readily be prepared with the same biochemical properties as those obtained in 0.88 M-sucrose, although they no longer stain with Janus green B and are spherical rather than elongated in shape. For the separation by differential centrifugation of the subcellular fractions from a homogenate in 0.25 M-sucrose prepared in a Potter-Elvehjem homogenizer, a speed of 700 g is employed to remove nuclei and general cell debris including unbroken cells. After removal of the nuclear fraction, the extract is centrifuged at 8,500 g for 10 minutes to bring down the mitochondria and at 57,000 g for 60 minutes to bring down the microsomes as an amber pellet. The supernatant fraction is said to be derived from the cell sap and contains no sedimentable material.

Although clean nuclei can be prepared by working up the nuclear fraction obtained in the procedure outlined above, more satisfactory preparations can be produced by homogenizing the tissue in citric acid (see Dounce (1955) for details) or in 0.25 M-sucrose containing 0.0018 M-calcium chloride.

Although Claude's original procedure or later modifications thereof involved the sedimentation at high speeds ($>$ 20,000 g) of particles of diameter 60–150 mμ., which were too small to be resolved by the light microscope, these particles were essentially "cytochemical concepts without any known morphological counterpart in the intact cell" (Palade & Siekevitz, 1956). The term microsomes was originally applied by Claude to these small granules, and recent work has made it clear that they consist of the disrupted remains of the endoplasmic reticulum. In a recent electron-microscope study of sections of the microsome pellet from liver tissue, Palade & Siekevitz (1956) have demonstrated that the predominant structural element is represented by membrane-bound profiles recalling those found in the endoplasmic reticulum in sections of the intact liver cell. These profiles appear to correspond in three dimensions to tubules or cisternae and may be smooth surfaced though the majority carry small dense particles 10–15 mμ. in diameter attached at the surface of the membrane. The microsomes therefore are not artifacts introduced by homogenization of the tissue but, as fragments of the endoplasmic reticulum, they represent cytoplasmic structures known to pre-exist in the intact cell.

When suspensions of microsomes are treated with sodium deoxycholate, they are disrupted into an unsedimentable portion derived from the membranous component and containing most of the protein and nearly all of the phospholipid, pigment and enzymes, and a particulate portion sedimentable at 105,000 g containing almost 20% of the protein and nearly all of the RNA of the microsomes, which is itself the bulk of the RNA of the cytoplasm. These small particles contain approximately equal amounts of RNA and protein (Littlefield, Keller, Gross & Zamecnik, 1955) and appear to correspond to the ribonucleoprotein macromolecules of Petermann *et al.* (1953).

3. Properties of the Subcellular Fractions

Hepatocytes, on account of their high relative cytoplasmic volume (the nucleocytoplasmic ratio is about 0.08), are probably more convenient than any other cell for the study of the subcellular fractions, and most of the information about their biochemical function has been obtained from liver tissue.

The mitochondria, of which there are about 400 in each liver cell, account for about 17% of the total cellular nitrogen and less than 5% of the total RNA. They contain all the cytochrome oxidase and succinoxidase activity of the cell and are characterized by their ability to oxidize many substrates, including fatty acids and intermediates of the Krebs citric acid cycle[1], as well as by their power of transforming the energy so released into the high-energy phosphate bonds of adenosine-triphosphate (ATP). As the seat of oxidative phosphorylation, they are sometimes referred to as the power house of the cell, but this statement is almost certainly a serious oversimplification, since the fact that whole cytoplasm is much more effective than mitochondria alone in catalysing some of the Krebs cycle oxidations indicates the existence of complicated cytoplasmic interactions about which little is known. The microsomes, on the other hand, contain about one-quarter of the total nitrogen of the cell and more than half of the total RNA. Most of this RNA is located in the small particles which are released from the microsomes by sodium deoxycholate treatment and which have a RNA : protein ratio approaching unity. Liver microsomes contain a high proportion (40% dry weight) of phospholipid together with a characteristic haemochromogen designated "cytochrome m" by Strittmatter & Ball (1954), and they are especially rich in the enzyme DPNH-cytochrome *c* reductase. Microsomes incorporate isotopically labelled amino acids into their proteins more rapidly than do any other cell fractions, and this phenomenon is most pronounced in their RNA-rich particles. It is probable that it is in these particles that RNA mediates in the process of protein synthesis in the cell (Zamecnik, 1956).

The cell sap, which contains the soluble proteins of the cell, accounts for about 40% of the total nitrogen of the cell and for about one-third of the RNA. By virtue of its content of the enzymes aconitase and *iso*citric dehydrogenase it participates in the reactions of the Krebs citric acid cycle, but it also contains more than 90% of the adenosine deaminase and nucleoside phosphorylase activity of the liver.

While it is customary to divide the subcellular particles into the two categories of mitochondria and microsomes, it should be kept in mind that both these fractions are capable of further subdivision on the basis of biochemical properties. Paigen (1954) and Kuff & Schneider (1954), for example, separated liver mitochondria by differential centrifugation into four groups with distinct enzyme complements, while de Duve and his colleagues (de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955) made an exhaustive study of the distribution of enzymes in liver tissue from which microsomes and two types of mitochondria had been prepared. Kuff, Hogeboom & Dalton (1956) separated from liver homogenates three types of cytoplasmic particulate component: (i) those of average radius 220–260 mμ. corresponding to the mitochondria and containing all the succinic dehydrogenase; (ii) those of radius 120 mμ. corresponding to vesicular structures derived from the endoplasmic reticulum and containing all the uricase activity and 26% of the RNA; and (iii) those of 25-mμ. dimensions containing over 50% of the RNA of the cell and of the DPNH-cytochrome *c* reductase activity.

The cell nucleus contains deoxyribonucleic acid (DNA), a characteristic component of the chromosomes. The mean amount of DNA per nucleus, as determined by chemical analysis of nuclei in bulk, is approximately constant for the diploid nuclei of all the tissues of a given species, although it varies widely between one species and another (for references see Vendrely, 1955). Haploid nuclei in sperm cells contain half this amount. Most rat tissues, e.g. kidney, contain

[1] See Krebs, *Brit. med. Bull.* 1953, 9, 97.—Ed.

about 0.65 pg. (1 picogram (pg.) = 10^{-12} g.) DNA phosphorus per nucleus, but liver tissue shows the much higher value of about 0.91 pg. (Thomson, Heagy, Hutchison & Davidson, 1953). This is due to the presence of many tetraploid nuclei with twice the normal diploid DNA complement as determined by estimations of DNA by spectrophotometric methods on individual nuclei (Swift, 1950; Frazer & Davidson, 1953).

Such polyploidy is also found in the human liver. Swartz (1956) has found that up to the age of six the human liver contains only diploid nuclei but that a definite tetraploid class is developed between the years 11 and 14 and is well established at the age of 20. At this age also, octoploid nuclei make their appearance and all three classes co-exist in the liver for the rest of the life-span.

In the rat, hypophysectomy does not alter the DNA content per nucleus within a given class of ploidy, but treatment of the hypophysectomized rats with pituitary growth hormone causes a transient increase in DNA per nucleus followed by a return to the control level (Di Stefano, Diermeier & Tepperman, 1955). In the livers of hypophysectomized rats the diploid and tetraploid ratios are higher than in controls, but the normal distribution pattern is restored by administration of growth hormone. Hypophysectomy also causes a reduction in the protein and RNA content of the liver cell which is made good by administration of growth hormone.

Thomson *et al.* (1953) have shown that, for rats of the same age, sex and initial body-weight, drastic alterations in the diet such as fasting, or the administration of a protein-free diet, cause no statistically significant changes either in the total amount of DNA per liver or in the mean amount of DNA per liver nucleus. Consequently it is possible to use DNA as a reference substance in terms of which the composition of a tissue may be expressed as amounts of a constituent per unit of DNA or per cell (Davidson & Leslie, 1950a, 1950b).

4. Composition of Liver Tissue

The results shown in Table I for the livers of fed and fasted rats show that a fast of 48 hours is followed by a sharp rise in DNA concentration, owing to loss of lipid and stored protein resulting in an increased number of cells per unit weight of liver tissue. There is also a rise in the concentration of LP, PN and RNAP. These figures may easily be misleading, since the absolute amounts of DNA per liver do not alter on fasting, while those of the other constituents fall. This is clearly shown when the mean amounts per cell or per unit of DNA are calculated (Table I).

Liver is in some ways a peculiarly complicated tissue for calculations on a per cell basis since, in addition to polyploidy, it shows the presence of many binucleate cells. Harrison (1953a, 1953b) has devised a useful method of calculation to allow not only for polyploidy but also for binucleate cells and for extracellular tissue in the calculation of the composition of the mean liver cell; some of her results are shown in Table II. Among the most striking features are the differences between the sexes in the content of iron and neutral lipids.

5. Influence of Dietary Changes

The composition of the liver can be greatly changed by alterations in the diet. The liver is of course an important storehouse of glycogen but it also contains labile protein. Addis, Poo & Lew (1936a, 1936b, 1936c) found that when rats were fasted for seven days the liver lost 40% of its initial protein content—a much higher proportion than was lost from any other tissue. This loss of protein is evident in the data presented in Tables I and II. Table II, which presents the results on a per cell basis, also shows the very striking fall in glycogen on fasting, while other cell constituents such as iron do not diminish, and neutral lipids actually increase in the female liver cell. Kosterlitz, in an extensive series of investigations in Aberdeen (see Kosterlitz & Campbell, 1945; Campbell & Kosterlitz, 1948a, 1948b, 1950, 1952; Kosterlitz, 1951), observing that fasting or the institution of a protein-free diet caused a concomitant loss of protein, phospholipid and RNA, applied the term "labile liver cytoplasm" to the material which disappeared. This is appropriate since the changes which occur are manifested essentially in the

TABLE I. EFFECT OF A 48-HR. FAST ON THE COMPOSITION OF RAT LIVER TISSUE IN TERMS OF DEOXYRIBONUCLEIC-ACID PHOSPHORUS (DNAP), LIPID PHOSPHORUS (LP), PROTEIN NITROGEN (PN) AND RIBONUCLEIC-ACID PHOSPHORUS (RNAP) (Davidson, 1954)

Condition of rat	DNAP	LP	PN	RNAP	Tissue mass
			mg./100 g. liver		
Fed	21	111	2,380	92	—
Fasted	33	140	2,603	108	—
			mg./liver		
Fed	1.7	9.0	193	7.5	—
Fasted	1.8	7.7	146	5.9	—
			pg.*/pg. DNAP		
Fed	1	5.3	116	4.4	4,810
Fasted	1	4.2	79	3.3	3,080

* Picogram (pg.) = 10^{-12} g.

TABLE II. COMPOSITION OF THE RAT LIVER CELL (after Harrison, 1953a, 1953b)

Constituents	Fresh tissue (fed rats) (g./100 g.)		Average amount in pg./diploid cell			
			(fed rats)		(rats fasted 6 days)*	
	Male	Female	Male	Female	Male	Female
Water	69.3	70.6	1,250	1,030	770	710
Protein	18.0	18.3	435	356	339	268
Glycogen	4.3	4.0	117	87	15	6
Neutral lipids	1.5	0.6	41	13	40	85
Phospholipids	2.8	2.7	76	59	57	50
RNA	0.96	1.08	26	24	20	17
DNA	0.21	0.26	6	6	6	6
Iron	0.017	0.046	0.35	0.96	0.41	1.01
Copper	0.00042	0.00052	0.011	0.012	0.011	0.010
Zinc	0.0042	0.0036	0.11	0.08	0.12	0.16

* During the 6-day fast the body-weight of the male rats fell from 271 g. to 210 g. and the liver weights fell from 8.91 g. to 5.41 g.

cytoplasm rather than in the nucleus—as can be demonstrated both on chemical grounds (Davidson, 1945; Kosterlitz, 1947; Thomson *et al.* 1953) and on histological grounds (Kosterlitz, 1947; Davidson, 1947). A similar loss of protein and RNA has been observed in the livers of severely malnourished human infants (Waterlow & Weisz, 1956; see also article by Waterlow & Bras in this number of the Bulletin, p. 107).

In the rat liver, the loss of cytoplasm involves a uniform loss of protein from mitochondria, microsomes and cell sap, a loss of RNA from the microsomes only and a loss of phospholipid from microsomes and cell sap (Wikramanayake, Heagy & Munro, 1953; Munro, 1954). The microsomes therefore appear to be most seriously affected (see also Muntwyler, Seifter & Harkness, 1950) and, since they are almost certainly the site of protein synthesis in the cell (Siekevitz, 1952; Zamecnik, 1956), it might be expected that protein depletion would be accompanied by defective protein synthesis. Munro, Naismith & Wikramanayake (1953) have, however, shown in tracer studies with ^{32}P that the reduction in total amount of liver RNA caused by removal of protein from the diet is counterbalanced by an increased rate of turnover in the remaining molecules, so that the total amount of RNA synthesized remains the same as before. Whereas the amount of RNA in the liver is influenced by protein intake, its rate of incorporation of ^{32}P is independent of this factor and is determined by energy intake. Thus, on a diet devoid of protein, increments in energy intake increase the turnover of RNAP, although they cause little change in the total amount of RNA and protein. This effect of energy intake on a protein-free diet affects all parts of the cell, including the microsomes (Wikramanayake, Heagy & Munro, 1953). There is accordingly no reason to believe that the capacity of the liver cell to form RNA is a factor limiting the rate of protein synthesis during protein depletion.

In 1953 Munro & Naismith showed that the total amount of protein in the liver varied linearly with energy intake in the form of carbohydrate or fat when the diet provided adequate amounts of protein; however, addition of energy to a protein-free diet tended to reduce the amount of liver protein. Comparison of the changes in the liver with those occurring in other tissues showed that energy intake, like protein intake, had a greater effect on the protein content of the liver than on the protein of other tissues generally. The addition of 1,000 cal./m.^{2} body-surface area caused a 23% increment in liver nitrogen, whereas the other viscera and the carcass underwent a change of only 3%.

The phospholipid content of the liver, like the RNA content, falls on a protein-free diet (Campbell & Kosterlitz, 1948b, 1952), and the reduction in phospholipid is accompanied by a compensatory increase in phosphorus uptake (as measured with ^{32}P) similar to that occurring with RNA, but the rate of synthesis declines after a few days of protein deficiency. At this stage increments in energy intake fail to augment the total rate of phospholipid synthesis, although synthesis on a diet containing protein increases under the same circumstances (Wikramanayake, Munro, Naismith & Hutchison, 1953).

The fluctuations in the protein content of the liver cell brought about by alterations in the diet are reflected in changes in the enzyme activity of liver tissue (Levy, 1953). Although it might be expected that those enzymes situated in the most labile parts of the cytoplasm would be most sensitive to protein depletion, no such regular pattern is in fact found. For example, xanthine oxidase which is located exclusively in the cell sap disappears completely (Meikleham, Wells, Richert & Westerfeld, 1951), while esterase activity decreases to 30% of its original value although it is found mainly in the particulate components of the cytoplasm. Most enzymes disappear in proportion to the amount of protein lost.

Munro (1954) has pointed out that the failure to locate a discrete fraction of the liver protein to which dietary variations are confined argues against the existence of a specific fraction of storage protein in the liver comparable, for example, to the stocks of glycogen, and suggests that apparent lability of the liver proteins by comparison with those of other tissues can be explained on the basis of the greater rate of turnover in the liver. The stability of the liver RNA, the amount of which is determined by the protein intake through regulation of the rate of RNA breakdown, is related in some way to the intensity of protein synthesis (Clark, Naismith & Munro, 1957). While synthesis of RNA is determined by the supply of energy, the amount of RNA will vary in accordance with changes in the rate of its breakdown in response to variations in the supply of amino acids.

6. Conclusion

The liver is a cytologically heterogeneous organ which carries out many functions in the body, but by the application of new techniques much information is now being obtained concerning the fine structure of the predominant type of liver cell, the hepatocyte. This increased knowledge of structure is being steadily correlated with our advancing understanding of the functions of the subcellular components both in the normal and in the diseased state.

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THE BLOOD FLOW OF THE LIVER

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1. Liver Pathology and Anoxia

In this paper the anatomy and physiology of the hepatic blood vessels is briefly considered, together with the function of these vessels in liver physiology. Because the liver has two different sources of blood, studies on the relationship of blood flow to hepatic function have proved surprisingly difficult, and much remains to be solved. Gross decrease of blood flow through the liver leads to anoxia and therefore to degenerative or necrotic changes. It has been shown that the development of necrosis, centrilobular at first but later spreading to mid-zonal and even periportal regions of the lobule, is largely due to hepatic anoxia and may be prevented or decreased by increasing the amount of oxygen in inspired air (Resnik & Keefer, 1926; Stander, 1926; Campbell, 1927; MacCallum, 1928; Rich, 1930; Ravdin, Eliason, Coates, Holloway, Ferguson, Gill & Cook, 1937; Goldschmidt, Ravdin & Lucké, 1937; McIver & Winter, 1943; Glynn & Himsworth, 1948; Himsworth, 1947; Delorme, 1951). Changes of function due to anoxia have not been fully investigated, but lack of oxygen may cause alterations of serum proteins (Rappaport, Clark & Stewart, 1952), and experimentally severe ischaemia can lead to hepatic coma in the presence of cirrhosis (Rappaport, Macdonald & Borowy, 1953). In experimental haemorrhagic shock the incidence of fatalities may be greatly reduced by keeping the hepatic blood well oxygenated, possibly by enabling the liver to metabolize vasodilator substances formed elsewhere in the body (Frank, Seligman & Fine, 1946; Delorme, 1951; Shorr, Zweifach, Furchgott & Baez, 1951). Anoxia, coupled with a high venous pressure, can give rise to watery vacuolation of the liver and disruption of the biliary canaliculi (Trowell, 1946; Grafflin & Bagley, 1952). The function of the liver may therefore be modified by the degree of oxygenation permitted by vascular regulation. In addition, the hepatic artery and portal vein of most species appear to have specific functions.

The bibliography on the hepatic vessels is so voluminous that only reviews and a few original articles are mentioned below. A general account of the subject has been given by Child (1954), and the following references deal with the technique and results of hepatic artery ligation (Graham & Cannell, 1933; Berman & Hull, 1953; Desforges, Campbell & Robbins, 1953; Ramström, 1953; Rienhoff & Woods, 1953).

2. Anatomy of the Intra-Hepatic Blood Vessels

The arrangement of the larger hepatic vessels in man varies considerably, making surgical anatomy difficult (Segall, 1923; Elias & Petty, 1952; Healey, Schroy & Sorensen, 1953). There is no anastomosis between portal venous branches of different portal tracts and, in man, apart from arterioles in the capsule, the vascular beds of the left and right sides of the liver are separate (McIndoe & Counseller, 1927). Glauser (1953) describes hepatic arterial branches as "end arteries", but this view is not generally accepted (Segall, 1923; Andrews, Maegraith & Wenyon, 1949; Michels, 1953; Rappaport, Lotto & Lougheed, 1954).

a. Portal Vein

Accounts of the microscopical anatomy are given by Mall (1906), McIndoe (1928), Andrews *et al.* (1949), Elias & Popper (1955). In man the largest branches are accompanied by a number of smaller branches, and the parenchyma is supplied via the accompanying and short direct branches. Smaller portal veins send branches direct to the parenchyma and terminate by flowing into several large sinusoids. Blood from the portal veins appears to flow firstly into a network of large sinusoids which surrounds the portal tracts and thence into the lobules (Andrews *et al.* 1949). Mixing of arterial and portal blood probably takes place at the periphery of the lobule, but Wakim (1944) stated that some sinusoids are arterial and others venous in their supply.

There are numerous connexions between the portal vein and a plexus of vessels which surrounds the intra-hepatic bile ducts: another vessel, acting as a small portal vein, drains this peribiliary plexus into the sinusoids. According to Elias & Popper (1955), the parenchyma around large portal veins is well supplied with portal blood in man and dog, but not in the rat. Stream-lining is present in portal blood, and blood from different parts of the splanchnic viscera tends to drain into definite parts of the liver. The greater vulnerability of the left lobe to nutritional changes, and of the right lobe to abscess formation, is thereby explained (Himsworth, 1947; Benz, Baggenstoss & Wollaeger, 1952).

b. Hepatic Artery

This artery supplies Glisson's capsule, structures within the portal tract, and the sinusoids (Olds & Stafford, 1930). On anatomical and physiological evidence, the presence of arterial branches direct to the hepatic venous tree has been described by Andrews & Maegraith (1953), but in the dog these probably carry only a small fraction of the total arterial flow at normal flow-rates (Andrews, Groom & Richards, to be published). More than one arterial branch is often present in a portal tract, and the branches may anastomose.

Arterial twigs often join portal venous branches as they leave the portal tract. Metastases of neoplasms in the liver receive purely an arterial supply (Wright, 1937).

c. Sinusoids

After injecting hepatic vessels with latex, the parenchyma may be digested away, leaving the sinusoids filled as irregular tubes. Liver cords consisting of a double string of parenchymal cells surrounded by blood do not therefore exist, and Elias (1949) has described the parenchyma as a series of anastomosing plates, between which run the sinusoids. The sinusoids are probably non-contractile, and their calibre is mostly dependent upon the amount of blood within them but may be reduced by swelling of parenchymal or reticulo-endothelial cells (Himsworth, 1947).

d. Hepatic Vein

In small animals, sinusoids drain into the central veins and also into the sublobular veins but, as the size of the hepatic venous vessel increases, the number of sinusoids entering direct decreases: many sinusoids are gathered into short trunks before entering the sublobular veins. These trunks correspond to the small sluice channels of Deysach (1941), constriction of which holds blood back in the lobules. In man, according to Elias & Popper (1955), the central veins alone receive sinusoids, and the sublobular veins are joined by central and sublobular veins only. Only the hepatic vein of the dog and racoon is said to contain smooth muscle (Arey, 1941). Andrews, Maegraith & Ritchie (unpublished observations), using lissamine-tartrazine staining, have demonstrated smooth muscle in the hepatic venous tree of man and several species of animal, extending from the larger branches into those 2 mm. or less in diameter; Thomas & Essex (1949) also found evidence for hepatic venous muscle.

e. The Vessels in Cirrhosis

The regular distribution of the vessels is completely distorted in cirrhosis, and the hepatic artery supplies a larger proportion of the blood. Connexions between the artery and vein become larger: this may be part of the aetiology of portal hypertension. In portal cirrhosis every lobule is distorted, and the blood supply to the parenchyma is irregular and frequently greatly reduced, the portal supply being more reduced than the hepatic arterial supply (McIndoe, 1928; Dock, 1942; Daniel, Prichard & Renell, 1952a; Popper, Elias & Petty, 1952).

3. Reactions of Hepatic Blood Vessels

In all species studied, the hepatic artery and portal vein are constricted by adrenaline, noradrenaline and sympathetic stimulation (Andrews, Hecker, Maegraith & Ritchie, 1955; Andrews, Hecker & Maegraith, 1956; Daniel & Prichard, 1951a; McMichael, 1932; Bauer, Dale, Poulsson & Richards, 1932). Reaction to other substances has not been extensively studied. Acetylcholine may constrict the portal vein of some species, but seldom of the dog and, when the hepatic artery is constricted by adrenaline, dilatation may be produced by acetylcholine or histamine (Andrews, Hecker, Maegraith & Ritchie, 1955; Andrews, Hecker & Maegraith, 1956).

In all species studied, adrenaline and noradrenaline can cause constriction of the hepatic veins, but in dogs they can also produce dilatation when the liver is congested: histamine constricts the hepatic veins of dog and monkey, though rarely of other species (Bauer *et al.* 1932; Andrews, Hecker, Maegraith & Ritchie, 1955; Andrews, Hecker & Maegraith, 1956). Yet the hepatic veins of several species may constrict in anaphylaxis, small and large branches reacting (Weil, 1917; Simonds & Brandes, 1927; Maegraith, Andrews & Wenyon, 1949; Andrews, Hecker, Maegraith & Ritchie, 1953). The constriction of sensitized hepatic veins may be of importance in allowing organisms such as *Entamoeba histolytica* to gain purchase in the liver (Maegraith & Harinasuta, 1954).

Blood flow through the sinusoids is said by American workers to be intermittent (Knisely, Bloch & Warner, 1948; Wakim & Mann, 1942). Hecker (1954) was unable to confirm this and, after injection of fluorescent substances, all the parenchymal cells are found to fluoresce within a few minutes (Grafflin & Bagley, 1952). Carbon tetrachloride produces swelling of parenchymal cells which, according to Himsworth (1947), may slow the sinusoidal blood flow. While Daniel, Prichard & Renell (1952b), using radiological methods, were unable to demonstrate such slowing, Daniel & Prichard (1951b) did show that under certain conditions the blood from the portal vein may be restricted to hilar regions. This finding may explain the observation of Andrews & Glockling (1956) that the degree of oxygenation of hepatic venous blood was not necessarily the same as that in peripheral sinusoids.

Although the direct effect of adrenaline and noradrenaline on the hepatic blood vessels is to reduce blood flow, adrenaline when administered into the general circulation of intact animals or man usually increases the flow greatly, whilst noradrenaline has little effect (Grayson & Johnson, 1953; Bradley, Ingelfinger & Bradley, 1952; Bearn, Billing & Sherlock, 1951). The augmented flow after adrenaline administration has been shown in rats to be largely reflex and initiated by aortic and carotid sinus receptors. The reflexes are abolished by coeliac neurectomy, administration of hexamethonium salts or atropine, and by deep anaesthesia (Ginsburg & Grayson, 1954).

4. Function of Hepatic Blood Vessels and Effects of Vascular Obstruction

The literature up to 1950 has been reviewed by Himsworth (1947, 1950), but further advances have been made during the last few years.

a. Portal Vein

In animals, shunting of portal blood into the inferior vena cava leads to atrophy and degeneration of the parenchyma (Whipple & Hooper, 1916; Bollman, 1948), but man appears to tolerate the procedure well, though admittedly the operation is only performed in the presence of an abnormal liver (Walker, 1954). Obstruction of the portal vein leads to atrophy, degeneration and occasionally necrosis, the lesion being primarily centrilobular (Rous & Larimore, 1920; Bywaters, 1946; McMichael, 1937), though Popper, Jefferson, Hassan, Phillips, Proffitt & Nicheles (1955) in short-term experiments did not find this degeneration in dogs. Portal venous blood may be necessary for parenchymal regeneration, but can be replaced to a large extent by diverting blood into the portal vein (Harkness, 1957; Fisher, Russ,

Fedor, Wilde, Engstrom & Fisher, 1955; Child, Barr, Holswade & Harrison, 1953). The portal vein supplies some of the oxygen required by the liver, the proportion supplied being differently given by various workers (McMichael, 1937; Blalock & Mason, 1936; Barcroft & Shore, 1912).

b. Hepatic Artery

The function of the hepatic artery almost certainly varies in different species. In the rat there is but a poor arterial supply to the bile duct, and ligature of the artery appears to be almost without effect on liver physiology (Wiles, Schenk & Lindenberg, 1952). Markowitz & Rappaport (1951) have shown that, after arterial blood is excluded from the liver of dogs, gas gangrene organisms develop, but this may not occur in other species (Cameron & Mayes, 1930); From & Alli (1956) have cultured 101 consecutive biopsy specimens from man without demonstrating the presence of such organisms, and even in dogs the importance of the organism is not clear (Eze, 1952).

In general, ligature or obstruction of the hepatic artery leads to centrilobular necrosis in man (Parker, 1955 ; Edgecombe & Gardner, 1951; Wooling, Baggenstoss & Weir, 1951; Bywaters, 1946), and in animals (Popper *et al.* 1955; Cameron & Mayes, 1930). Ligature of the hepatic artery, but not total deprivation of the liver of arterial blood, has been used to reduce portal hypertension in man (Smith, McNealy & Zaus, 1953; McFadzean & Cook, 1953; Berman, Koenig & Muller, 1951).

Andrews, Maegraith & Richards (1956) have shown that, in dogs, sulphobromophthalein (formerly known as bromsulphalein) is removed more rapidly from hepatic arterial than from portal venous blood: later Andrews & Richards (to be published) have shown that biliary excretion of this dye occurs from the hepatic arterial bed only. Replacing portal venous blood by arterial blood does not affect the removal of sulphobromophthalein from blood of dogs (Fisher *et al.* 1955). That the liver of man is probably similar to that of the dog in this respect may be deduced from the figures of Bradley *et al.* (1952). Andrews (1955) has suggested, on this and other evidence, that the bile ducts secrete at least some constituents of bile, and that biliary function is dependent upon an hepatic arterial flow; this does not apply, however, to the rat (Brauer, Leong & Holloway, 1954).

c. Hepatic Vein

Obstruction following local inflammation of the hepatic veins in man was first described by Chiari (1899), and numerous cases have now been reported (Thompson & Turnbull, 1912; Fruhling, Roger & Jobard, 1951); a similar condition, possibly due to senecio poisoning, has been described by Bras, Jelliffe & Stuart (1954) under the title of veno-occlusive disease.[1] The typical lesion of hepatic venous obstruction is that of centrilobular parenchymal necrosis or degeneration with congestion, both in man (references above) and animals (Bolton & Barnard, 1931; Simonds & Callaway, 1932).

Centrilobular congestion is almost invariable in lesions of the liver (see, for example, Cameron & Mayes, 1930; Cameron & Karunaratne, 1936; Popper *et al.* 1955) and is common in shock (Ellenberg & Osserman, 1951). Maegraith and his colleagues (Maegraith & Findlay, 1944; Maegraith, Andrews & Gall, 1947) suggested that, in shock, active constriction of the hepatic veins may take place through reflex action. The venous constriction would produce congestion and some degree of stagnant anoxia which, if severe and prolonged and especially if other factors such as anaemia or pyrexia were present, would result in hepatic centrilobular degeneration.

Although not proved, Maegraith's attractive hypothesis is supported by the available evidence, which he has summarized (Maegraith, 1948). In addition, active constriction of hepatic veins can take place in man, since spasm may occur during catheterization (Bradley, Ingelfinger, Bradley & Curry, 1945), and enlargement and regression of the liver may take place within a few hours (Beattie, Herbert, Wechtel & Steele, 1944). Congestion of the liver is frequently present in shock, despite the reduced caval pressure, and vascular obstruction to hepatic blood flow occurs in haemorrhagic shock in dogs (Wiggers, Opdyke & Johnson, 1946), though not in rats (Friedman, Milrod, Frank & Fine, 1953). A condition in piglets which is apparently due to reflex hepatic venous constriction is described by Naftalin & Howie (1949), and evidence in favour of the presence of nerves is given by Andrews, Hecker & Maegraith (1954). The pathways of nerve impulses have not been described, but they may be similar to those of the caval sphincter present in seals (Harrison, Tomlinson & Bernstein, 1954).

5. Total Hepatic Blood-Flow Measurements

Measurements of total hepatic blood flow without surgery are now possible. For small animals the method of Grayson (1952), using a modified thermostromuhr, has proved a useful tool, but for relative rather than absolute flow-rates.

The method of Bradley (Bradley *et al.* 1945) is commonly used for estimating hepatic blood flow in man and dogs. The method depends upon the uptake of sulphobromophthalein by the liver: by giving a continuous intravenous infusion and using the Fick principle, a fair degree of accuracy is obtained, though inaccuracies undoubtedly exist (Selkurt, 1953). The method gives the estimated hepatic blood flow (EHBF) in man as approx. 1,500 ml./min., the flow being related to the body-surface area. Other workers using the same method obtain similar values (for references see last paragraph). Galactose has been used, with the Fick principle, for measurement of the estimated hepatic blood flow (Hansen, Tygstrup & Winkler, 1954), and the results were similar to those obtained by Bradley *et al.* (1945). Colloidal particles are removed almost entirely by the reticuloendothelial cells of liver and spleen, and if they are radioactive the rate of disappearance of radioactivity from the blood may be used to estimate hepatic blood flow. Colloidal chromic phosphate, containing ^{32}P, and colloidal gold, ^{198}Au, give results comparable with those obtained with sulphobromophthalein, but variations in the haematocrit value of blood in different vessels may give rise to some difficulties in assessing EHBF (Dobson, Warner, Finney & Johnston, 1953; Vetter, Grabner, Höfer, Neumayr & Parzer, 1956).

Liver blood flow of man has been shown to increase after administration of adrenaline and after the pyrogenic reaction (Bradley *et al.* 1952). There is little change in flow in pregnancy (Munnell & Taylor, 1947) and in hyperthyroidism (Myers, Brannon & Holland, 1950), but a reduction in cardiac failure (Myers & Hickan, 1948), cirrhosis (Myers, 1950) and fainting (Bearn, Billing, Edholm & Sherlock, 1951).

[1] See also Waterlow & Bras, p. 110 of this number of the Bulletin.—Ed.

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REGENERATION OF LIVER

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1 Structural changes in regenerating liver
  a Parenchymal cells
  b Littoral cells, bile-duct epithelium and connective tissue cells
2 Reorganization of structure in regenerating liver
3 Composition of the liver during regeneration
4 Function in regenerating liver
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The remarkable ability of the liver to replace lost tissue rapidly and completely by compensatory cellular hypertrophy and hyperplasia has long been recognized. The earlier work (see Fishback (1929) for review) was sporadic, but since 1931, when Higgins & Anderson published a full quantitative account of the changes in weight of the liver of the rat following partial hepatectomy and described a simple method for this operation, a large scattered literature on liver regeneration, mainly concerning the rat, has grown up. Although some aspects of the subject have been considered (Mann, 1944; Himsworth, 1947; Cameron, 1952), the bulk of this literature has never been collected together and reviewed critically. Such a review, although needed, would be beyond the scope of the present article which will be confined primarily to a consideration of regeneration of liver as a problem of morphogenesis. It will not be possible to consider the more complex topic of liver regeneration after toxic damage.

In partial hepatectomy by Higgins and Anderson's method, which is almost universally used, the median and left lateral lobe are removed a few mm. distal to a single thread ligature tied round their pedicles. These two lobes together amount to about two-thirds of the liver weight. The operation appears to have little outward effect in the rat and there is no mortality if it is properly performed (Gurd & Vars, 1949; and my personal experience). A number of descriptions have appeared in the literature (Higgins & Anderson, 1931; Brues, Drury & Brues, 1936; Marshak & Byron, 1945; Crandall & Drabkin, 1946; Roberts & Szego, 1947; Gurd, Vars & Ravdin, 1948; Ralli & Dumm, 1951).

Partial hepatectomy quickly causes changes in the structure, composition, and function of the residual liver. The effects of partial hepatectomy on the liver of the rat may be summarized as follows: there is an initial period of 6–12 hours, during which changes resembling those of starvation in exaggerated form take place, together with some others but without much of the type of change which normally characterizes growth. Changes of this latter type become obvious, however, in the second half of the first day, e.g., increase in weight, increased incorporation of isotopic materials into deoxyribonucleic acid (DNA). These changes culminate in an outburst of mitotic division in the parenchymal cells 24–30 hours after operation. Changes in other types of cell and in connective tissues are slower to get under way. The liver returns on a roughly exponential course to approximately normal size and, in many but not all respects, to normal composition in 10–20 days. The effects of partial hepatectomy are similar in the mouse. No other animal has been studied in detail.

1. Structural Changes in Regenerating Liver

a. Parenchymal Cells

The parenchymal cells are by far the most conspicuous elements in the liver, and most studies of regeneration have concentrated on them. In the first day they swell (50–100%) in volume, cytoplasm, nucleus and nucleolus all being involved, though the relations between the time-courses of the increase in volume of the different components of the cell are not clearly established (rat—Ferreira, 1940; Stowell, 1948; Bucher & Glinos, 1950; Abercrombie & Harkness, 1951; Grampa & Marinoni, 1951; Hammarsten, 1951; Harkness, 1952a). The swelling of the cells is accompanied by other histologically detectable changes, e.g., increase in cytoplasmic ribonucleic acid (RNA) (Davidson & Waymouth, 1944), increase in cytoplasmic lipids, and rapid loss of glycogen. It culminates between 24 and 30 hours after operation in a burst of mitotic division, accompanied by division of binuclear cells so that the normally high proportion of these is reduced (rat—Beams & King, 1942; Bucher & Glinos, 1950; St. Aubin & Bucher, 1952; Sulkin, 1943; mouse—Wilson, Stowell, Yokoyama & Tsuboi, 1953). The bulk of the evidence indicates that, in the rat, cell division starts rather abruptly, so that although animals killed exactly 24 hours after operation may show none, maximum frequency is reached a few hours later, to decline subsequently (Brues & Marble, 1937; Landing, Seed & Banfield, 1949; Grampa & Marinoni, 1951; Hammarsten, 1951; Abercrombie & Harkness, 1951; Harkness, 1952a; Johnson & Albert, 1952).[1] The maximum frequency of cell division seems to be reached later in the mouse (Wilson *et al.* 1953). The report of Marshak & Byron (1945) that the time of maximum frequency varies with age and is reached later in older rats should be confirmed by another method, since mitotic counts were done on nuclei isolated by centrifugation, and the mitotic rates were very much lower than found by other methods. Comparison of evidence from nuclear counts and rates of mitosis shows that there is no need to postulate amitotic division. The calculated mean durations of mitosis are in the region of an hour (Brues & Marble, 1937; Landing *et al.* 1949). However, much more needs to be known about the duration of mitosis at different times after partial hepatectomy, and the above estimates will probably have to be modified in view of the interesting finding of Jaffe (1954) that there is a very large diurnal variation in rate of mitosis in regenerating liver, the rhythm corresponding to that of normal liver mitosis—i.e., high in the morning and low at night.

The result of this very active cell division is that the number of parenchymal nuclei in liver returns rapidly towards normal, though it lags all the time behind growth in weight (fig. 1). It returns finally to normal only very slowly if at all, and is

[1] The cells in mitosis tend to contain less glycogen and less fat than the non-mitosing cells (Ferrari, 1954; Ferrari & Brondoni, 1954).

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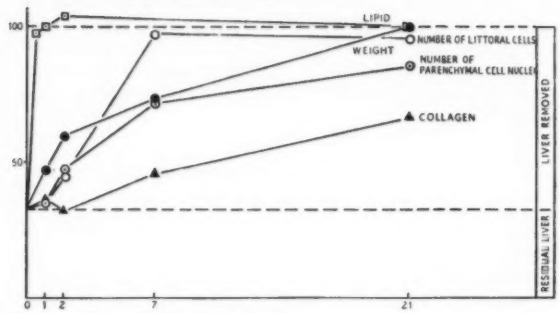
FIG. 1. THE RATES AT WHICH THE TOTAL QUANTITIES OF DIFFERENT COMPONENTS OF LIVER RETURN TOWARDS NORMAL AFTER PARTIAL HEPATECTOMY IN THE RAT



Abscissae: days after hepatectomy
Ordinates: total of different components of liver (as percentage of pre-operative total)

Extreme types of recovery curve are shown by the curves for lipid and collagen. Most other individual components would cluster about the curve for weight. Data from Abercrombie & Harkness (1951) and Harkness (1952a, 1952b).

still below normal after three weeks. This failure of multiplication to return the number of nuclei to normal is associated with an increase in average nuclear volume, as if the failure were not so much in increase of nuclear material as in nuclear division (rat—Biesele, 1944; Abercrombie & Harkness, 1951; mouse—Yokoyama, Wilson, Tsuboi & Stowell, 1953). The tendency to compensatory growth by increase in size rather than in number of cells appears to be exaggerated if more than two-thirds of the liver is removed. By removing the liver in several stages, allowing regeneration to take place between each stage, it is possible to get complete regeneration in weight from only one of the parts of the small caudate lobe (Forti, 1954). Besides the above changes, formation of various intracellular inclusion bodies in parenchymal cells has been described (Gurd & Vars, 1949; Doniach & Weinbren, 1952; Aterman, 1952).

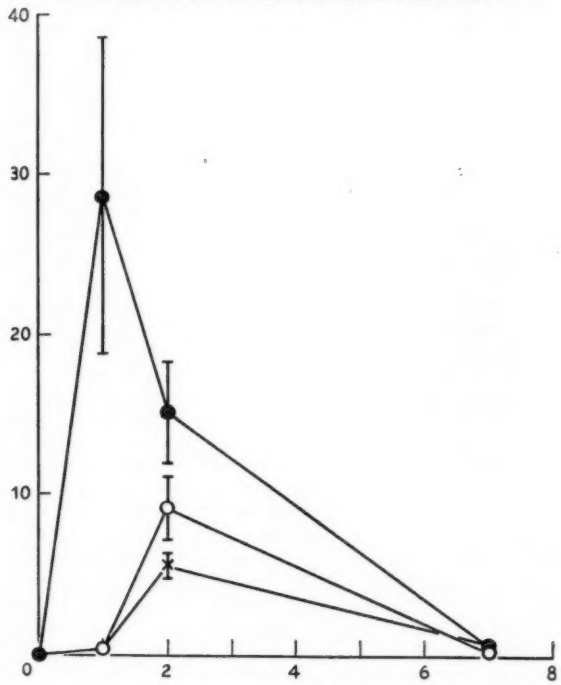
b. Littoral Cells, Bile-Duct Epithelium and Connective Tissue Cells

Very little is known about changes in cells other than parenchymal. Though these other cells occupy roughly only 5% or so of the volume of the liver (rat—Harkness, 1952a), they are much more important numerically, accounting for 30–40% of all the nuclei in the liver (Abercrombie & Harkness, 1951). Littoral cells show the same sort of changes as are found in the parenchymal cells, but the course of the changes appears to be delayed at first more than those of the parenchymal cells; for example, the maximal mitosis was found to be on the second day after partial hepatectomy, whereas in the parenchymal cells it was on the first day (fig. 2). Changes affecting the morphology of littoral cells at a much earlier stage of regeneration have been described (Aterman, 1952). A complication affecting the regeneration of littoral cells is introduced by the possibility of their increasing not only by mitosis but also by recruitment from blood. An increase in the proportion of cells of this type a week after partial hepatectomy has been described (Abercrombie & Harkness, 1951). Such an increase would explain some of the rather anomalous results of crude total counts on isolated nuclei (Price & Laird, 1950) and of measurements of deoxyribonucleic acid (Mills, Paul & Smith, 1953). Mitosis in peritoneal endothelium, connective tissue, cells of blood-vessel walls and bile-duct epithelium also starts later than in parenchymal cells and appears to follow the same course as in littoral cells (Abercrombie & Harkness, 1951). There is no evidence that bile-duct epithelium contributes to the regeneration of parenchymal cells; in fact, evidence from the time-course of mitosis is against this hypothesis.

2. Reorganization of Structure in Regenerating Liver

Judged by weight, growth is fairly even and equal in the different residual lobes (Harkness & Harkness, 1954). On the other hand, growth within the lobule is not even—for example, mitosis in parenchymal cells at least initially is mainly periportal in distribution (von Meister, 1894; Milne, 1909; Harkness, 1952b). Growth is by increase in the size of the individual lobules rather than in their number, and there is only a small increase in the number of bile ducts and

FIG. 2. MEAN NUMBERS OF MITOSES PER 1,000 CELLS AT DIFFERENT TIMES AFTER OPERATION



Abercrombie & Harkness (1951) by permission of the Royal Society

Abscissae: days after operation
Ordinates: mitoses/1,000 nuclei

Each point is the mean of the values for eight rats; the vertical lines represent ± one standard error of the mean.

●: parenchymal cells
O: littoral cells
X: bile-duct epithelium

centrilobular veins (Abercrombie & Harkness, 1951; Forti, 1954), i.e., the type of adaptation, by increase in size rather than number, is the same at this morphological level as at parenchymal cell level. And again at this level, connective tissue changes lag behind parenchymal. Extracellular space is encroached upon by the swelling parenchymal cells in the first day after partial hepatectomy (Stowell, 1948; Harkness, 1952a) and is only restored later. Vascular space, judged by haemoglobin estimation, expands even more slowly than extracellular space as a whole (Harkness, 1952a). Similarly the growth of collagen is very slow, and even six weeks after partial hepatectomy the concentration is still below normal (Harkness, 1952c; Harkness & Harkness, 1954). The intralobular reticulin fraction of the collagen seems to regenerate rather faster than the collagen of the larger vessels. The largest increase in collagen appears to be in the peritoneal capsule, possibly as a result of mild inflammation associated with operative interference. The connective-tissue framework appears to stretch very slowly to adapt itself to the growth of the cells (Harkness, 1957).

The changes in behaviour of cells which underlie the structural reorganization that takes place during regeneration have received little attention, though studies of tissue culture of explants from regenerating liver show a greater outwandering of cells from regenerating than from normal livers (Glinos, 1949; Glinos & Bartlett, 1951; Abercrombie & Harkness, 1951). All types of cell show increased activity. Parenchymal cells show generally less activity than others and tend to survive badly in culture, as in transplants (Cameron & Oakley, 1934), but, with macrophages, fibroblasts and bile-duct epithelium, increased tendency to outwander in vitro parallels increased mitosis in vivo.

3. Composition of the Liver During Regeneration

Little attempt has been made in investigations of the composition of regenerating liver to distinguish the site of origin of the various constituents, it being generally assumed that an origin other than from the parenchymal cells can be neglected. While this may be true for most constituents it is clearly not true for some—e.g. DNA—and may well not be true for others—e.g. enzymes. One can, however, distinguish to some extent cytoplasmic and nuclear constituents and extracellular constituents. The latter have already been discussed. Very rapid changes occur in cytoplasmic storage products such as glycogen and neutral fat. There is a rapid rise in total liver lipid (rat—Collip, Kutz, Long, Thomson, Toby & Selye, 1935; Bogetti & Mazzocco, 1939; Gurd *et al.* 1948; Szego & Roberts, 1949; Lombroso, 1950; Harkness, 1952a; mouse—Yokoyama *et al.* 1953); and most of this is neutral fat (Ludewig, Minor & Hortenstine, 1939). Glycogen shows a sharp fall to very low levels, and slow recovery (rat—Stone, 1935; Bogetti & Mazzocco, 1941; Novikoff & Potter, 1948; Szego & Roberts, 1949; Harkness, 1952a). Total liver protein drops in concentration, and the absolute amount in the whole liver does not begin to increase appreciably for 12 hours or so (rat—Brues *et al.* 1936; Bogetti & Mazzocco, 1939; Szego & Roberts, 1948; Gurd *et al.* 1948; Harkness, 1952a; mouse—Roberts, 1953; Yokoyama *et al.* 1953). After this it rises rapidly, though lagging behind weight so that concentration is below normal for some days. The maximum rate of increase per unit of protein already present appears to be between 12 and 24 hours after partial hepatectomy. The pattern in this case resembles that for cell division: a period of inertia, shorter in this case than before mitosis, followed by a rapid rise to maximum rate of increase, with subsequently an exponential type of course towards the final equilibrium. The individual enzymic components of the total protein, which are too numerous to refer to in detail, appear to follow a generally similar type of course with some exceptions, e.g. catalase (Stein, Skavinski, Appleman & Shugarman, 1951); alkaline phosphatase (rat—Oppenheimer & Flock, 1947; Sulkin & Gardner, 1948; Norberg, 1949; Rosenthal, Fahl & Vars, 1952; Goodlad, Mills & Smith, 1951; Fumagalli & Petrelli, 1954; mouse—Yokoyama *et al.* 1953). The latter enzyme rises rapidly in concentration, though it seems likely that this has little functional relation to the growth process, but is a result of relative deficiency of the excretory capacity of the liver (Rosenthal *et al.* 1952). In general, increase in mitochondrial enzymes is slower than in other enzymes (see Harkness, 1952a; Greenbaum, Greenwood & Harkness, 1954). This and other not entirely unconflicting evidence suggests that changes in the microsomes and soluble fraction of cytoplasm precede those in mitochondria (Price & Laird, 1950; Allard, de Lamirande & Cantero, 1952; Petermann, Mizen & Hamilton, 1953; Thompson & Moss, 1955; Weber & Cantero, 1955). Cytoplasmic pentosenucleic acid (PNA) shows a rapid rise to high levels of concentration (Stowell, 1948; Novikoff & Potter, 1948; Rapport, Canzanelli & Guild, 1948; Bergstrand, Eliasson, Hammarsten, Norberg, Reichard & von Ubisch, 1948; Drochmans, 1950; Hammarsten, 1951; Price & Laird, 1950; Johnson & Albert, 1952; Lombardo, Cerecedo & Reddy, 1953). Nuclear DNA shows little change for 12 hours or so, but rises by the time of onset of mitosis to approximately double the original level and subsequently follows a course not much different from that of weight (Stowell, 1948; Novikoff & Potter, 1948; Rapport *et al.* 1948; Bergstrand *et al.* 1948; Drochmans, 1950; Price & Laird, 1950; Hammarsten, 1951; Johnson & Albert, 1952; Lombardo *et al.* 1953).

In summary, so far as work on the composition of the liver after partial hepatectomy can be interpreted in morphological terms, it shows a picture similar to that obtained from direct morphological study. Variations in composition from normality are generally greatest in the early period 1–2 days after partial hepatectomy and they appear to be brought about by variations in the time reactions of the different components. The growth curves of different components have different time constants but seem to have common characteristics: i.e., a period of inertia varying from a few hours in the case of lipid to 2–3 days in the case of collagen, followed by a rapid rise to maximal rate, with subsequently an approximately exponential decline in rate as the final equilibrium position is approached. A conspicuous feature of work on the composition of regenerating liver is the large amount of attention which has been paid to this rather uninteresting period of declining rates of change, during which divergence from normal becomes smaller and smaller and more and more subject to extraneous factors whose primary action is upon the equilibrium state. The crucially important period preceding mitosis has been comparatively neglected.

4. Function in Regenerating Liver

Few direct investigations of liver function after partial hepatectomy have been made. Total urea output is reduced

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for about three days (Thompson & Moss, 1955); but this is presumably a reflection of the diminution in food intake which follows partial hepatectomy, and nothing is known of the maximum capacity of the liver to form urea. Bile output is initially depressed but returns a little faster to normal than the regeneration in weight of liver, though there is some dissociation between volume and cholic acid output (Fisher, Zerbe & Vars, 1953). Indirect evidence of liver function can be obtained from examination of blood. Plasma glucose seems little affected (Reinecke, Rudolph, Bryson & Samuels, 1948). Individual and total plasma amino-acid levels also seem little affected, though there is some conflict of evidence here (Desmarais & Dugal, 1948; Ferrari & Harkness, 1954). Blood urea concentration is little affected (Ferrari & Harkness, 1954). A short-lived rise in bile-pigment concentration has been recorded (Royer & Bogetti, 1939a, 1939b). A rise in plasma-free cholesterol and other changes in plasma lipids have been recorded (Chanutin & Ludewig, 1936; Royer & Bogetti, 1939a, 1939b; Chanutin & Gjessing, 1949a, 1949b). One most marked and consistent change is in the concentration of plasma proteins which falls, although the evidence on how the individual components change is not altogether consistent (Chanutin, Hortenstine, Cole & Ludewig, 1938; de Lamirande & Cantero, 1952; Roberts, 1953; Chanutin & Gjessing, 1949a, 1949b; Roberts & White, 1949; Emmrich & Petzold, 1953).

A number of investigations of liver function with isotopically labelled materials have been made. Investigations with labelled amino acids (Rittenberg, Sproul & Shemin, 1948; Eliasson, Hammarsten, Reichard, Åqvist, Thorell & Ehrensvard, 1951; Åqvist, 1951; Hammarsten, 1951; Moyson, 1955) show comparatively small changes in incorporation, maximal in the third day after operation, and not in the first when growth of protein per unit already present is greatest. (Moyson's work is particularly interesting in that he describes effects on parenchymal and littoral cells separately.) It has been suggested that this maximum may reflect an increase in plasma protein synthesis (Greenbaum *et al.* 1954), but the interpretation of these results is made difficult, if not impossible, by lack of information on the accessibility of the labelled material to the site of protein synthesis and on the sizes of the amino-acid pools concerned. In addition there is evidence that considerable changes in amino-acid concentrations occur in regenerating liver (Ferrari & Harkness, 1954). A great deal of work on the incorporation of labelled material into nucleic acids has been done, but this has been directed more to the problem of nucleic acid metabolism than to that of regenerating liver per se, and it will not therefore be reviewed here. (See Brues, Tracy & Cohn, 1942; Davidson & Raymond, 1948; Bergstrand *et al.* 1948; Eliasson *et al.* 1951; Furst, Roll & Brown, 1950; Reichard, 1950; Hammarsten, 1951; Johnson & Albert, 1952; Smellie, McIndoe, Logan & Davidson, 1953; Ultmann, Hirschberg & Gellhorn, 1953; Anderson & Åqvist, 1953; Crosbie, Smellie & Davidson, 1953; Stevens, Daoust & Leblond, 1953; Nygaard & Rusch, 1955.) From the point of view of this article the most interesting feature of these investigations is that they draw attention to cell division as a predominant characteristic of regenerating liver. Incorporation of labelled material into DNA is conspicuously increased and the evidence supports that from morphology in indicating a delay of some 12 hours before marked nuclear changes appear.

5. The Stimulus to Regeneration

Our ignorance about the basic problem of the stimulus to regeneration is still almost complete. Indeed it is not even clear whether the same factors control the complex series of changes involved in regeneration as control the variations in amount of liver substance which occur in the normal life of an animal under varying conditions. The striking feature of regeneration is the extensive cellular multiplication which takes place, but there is little evidence to show the extent to which cellular multiplication is involved in other circumstances in which the amount of liver substance varies.

Before discussing the nature of the stimulus to regeneration, it is of interest to consider first the question of where it acts. One would expect it to act upon the main functional elements—that is, the parenchymal cells—and evidence from the time relations of events in different types of cell supports this hypothesis. Parenchymal cells react first, and the general sequence of changes suggests that when they are activated they in turn activate the other elements.

In considering the identity of the stimulus to regeneration, it is simplest to start by putting aside certain possibilities as unlikely. A good deal of evidence is available from the effects of various experimental procedures on regeneration, though much of this is difficult to interpret because the criterion of regeneration has been equivocal (e.g., wet weight), or because of failure to distinguish between factors affecting regeneration and factors affecting the equilibrium size of the liver (for discussion of this point see Williams, 1951; Weinbren, 1955). It seems clear, however, that considerable variations in diet have little effect on the basic process of regeneration, which takes place even on a protein-free diet (Brues *et al.* 1936; Vars & Gurd, 1947; Drabkin, 1947; Rapport *et al.* 1948; Rogers, Ferguson, Friedgood & Vars, 1950; Arrigo & Pontremoli, 1950; Ferrari & Brondoni, 1954). A high-fat diet seems to reduce the rate of increase in parenchymal cells (Brues *et al.* 1936). Of particular interest is the finding that the increase in number of parenchymal cell nuclei is practically the same in starved as in fed rats, though the increase in weight of the liver is markedly less. It also seems clear that none of the endocrine glands which have been investigated forms a critical link in the chain of reactions which leads to liver regeneration (pituitary—Franseen, Brues & Richards, 1938; Higgins & Ingle, 1939; Houssay, 1948; Canzanelli, Guild & Rapport, 1949; Fumagalli, Petrelli & Petrelli, 1954; adrenals—MacKay & Carne, 1938; Berman, Sylvester, Hay & Selye, 1947; Canzanelli, Guild & Rapport, 1949; Arrigo & Pontremoli, 1950; Drabkin, 1950; Friedgood, Vars & Zerbe, 1950; Ferrari & Harkness, 1954; Einhorn, Hirschberg & Gellhorn, 1954; Thompson & Moss, 1955; thyroid—Higgins, 1933; Fogelman & Ivy, 1948; Canzanelli, Rapport & Guild, 1949; Drabkin, 1950; gonads—Szego & Roberts, 1949). Nor is the spleen involved (Higgins & Priestley, 1932). Accumulation of excretory products is also unlikely to be involved. Indeed their presence in increased local concentration induced by tying the bile ducts has an inhibitory effect on regeneration (Rous & Larimore, 1920; Cameron, 1935; Weinbren, 1955). On the other hand, the most marked effects of bile-duct ligation are seen when normal lobes of liver with intact biliary excretory system which can hypertrophy, are present; this suggests that the diversion of the bile to the unobstructed lobes may be involved in their hypertrophy. Complete obstruction of the main bile duct in the rat does not prevent regeneration

(Ferguson, Rogers, Vars & Zerbe, 1949). Changes in the composition of metabolites in blood are in most cases too small, as noted above, to make it likely they are involved.

From the work which has been done on this subject, two factors emerge as most likely to be intimately concerned in regeneration: the blood supply to the liver, and plasma protein concentration. These will be discussed separately.

6. The Blood Supply of the Liver, and Regeneration

The blood supply of the liver has for long been considered to play a fundamental role in the control of regeneration. Its importance in regeneration has been emphasized by Mann (see review, Mann, 1944). Interest was early focused on the portal vein by Rous & Larimore (1920) who showed that tying a branch of the portal vein in rabbits led to atrophy of the lobe supplied by it and to hypertrophy of the rest of the liver. It was also shown that some sort of competition existed between lobes, since tying the bile duct of the parts with intact portal supply reduced their hypertrophy and also reduced the atrophy of the deprived lobes.

The importance of the portal supply was further emphasized by Mann and his co-workers who produced evidence that shunting the portal flow away through an Eck's fistula reduced or prevented regeneration (Mann, 1944; Stephenson, 1932). No adequate evidence on the effect of deprivation of hepatic arterial blood was obtained, because it usually caused hepatic necrosis, though recently evidence has been obtained that ligation of the hepatic artery does not affect regeneration in the rat (Karn & Vars, 1951). On the basis of the experiments on portal deprivation, it was generally assumed that some specific factor concerned in liver maintenance was present in the portal blood, i.e., in effect, that regeneration was controlled by one of the organs' draining its blood into the portal stream; and that the stimulus did not spread into the general blood stream. Mann (1944) has laid emphasis on the importance of portal volume flow, though it is difficult to see how this could act per se. Recent work has shown that these ideas must be modified, and it is now quite clear that the changes in the blood concerned in regeneration are not confined to the portal vein but occur in the general circulation. Regeneration does in fact occur in the absence of a portal blood supply in the rat, if the experiment is made in such a way that the size which the deprived lobes eventually reach (the equilibrium size) is greater after than before partial hepatectomy (Weinbren, 1955). Partial hepatectomy has been found to cause regenerative changes in the liver of a parabiotic partner (Christensen & Jacobsen, 1949; Bucher, Scott & Aub, 1950, 1951; Wenneker & Sussmann, 1951). If the normal portal blood supply is replaced by the arterial or inferior vena cava supply, regeneration proceeds normally (Child, Barr, Holswade & Harrison, 1953; Fisher, Russ & Bluestone, 1955; Fisher, Russ, Updegraff & Fisher, 1954).

In brief, it appears that available blood supply determines equilibrium liver size and so may affect regeneration. Whatever the active factor, it is present in arterial as well as portal blood. It is generally assumed that the total volume flow of blood through the liver remains at roughly its pre-operative level after partial hepatectomy, so that volume flow per unit weight of liver is increased. But, apart from evidence (Daniel & Prichard, 1951) that radio-opaque material put into the portal circulation goes more quickly through the liver after partial hepatectomy, there is an almost total lack of quantitative information on blood flow through the liver after partial hepatectomy. Further, there is very little information on how volume flow is adjusted to metabolic demand in the normal liver, and without this information one cannot hope to go further in the problem of the relation of blood flow to regeneration. Blood flow is presumably related to the size of the vascular bed, which increases during regeneration.

7. Plasma Proteins and Regeneration

The plasma proteins form a major group of the products synthesized by the liver and, as noted above, signs of insufficient liver function are shown after partial hepatectomy by a fall in plasma protein concentration. Interest in this change is increased by the finding of Glinos & Gey (1952) that plasmapheresis causes mitosis in livers of normal rats; and recently Glinos (1956a, 1956b) has produced evidence that, conversely, the regenerative changes after partial hepatectomy can be inhibited by increasing plasma concentration and that the active fraction is the albumin. On the basis of these findings, Glinos has suggested that plasma albumin concentration controls regeneration.

This evidence indicates primarily that the stimulus to regeneration is the reduction in concentration of some constituent of plasma, and it seems clear that the constituent most likely to be involved is the protein. The available evidence implicates the albumin fraction particularly. There are, however, difficulties in this hypothesis. As reported in the literature, change in blood-plasma albumin concentration appears to be too slow to account for the rapidity of the onset of regenerative changes in the liver. However, Glinos (1956a) has produced suggestive evidence that change in the protein concentration in the immediate environment of the hepatic cells may occur earlier and independently of change in the plasma. Such extravascular change could be produced by increased pressure in the portal system, which is known to occur after partial hepatectomy (Grindlay & Bollman, 1952). This suggested control system for liver regeneration appears to be the first clearly defined one for which concrete evidence has been produced.

8. Conclusion

In this survey the subject has been examined mainly from an academic standpoint and, to make the picture of the problem complete, a good deal of emphasis has had to be laid on the gaps in knowledge. It is largely because these gaps are so large that it is difficult, as yet, to suggest any direct application of this work to clinical medicine, and no attempt has been made to do so.

The question of the stimulus to regeneration is of course the fundamental one, and progress on the morphogenic side of the problem ultimately depends on its being solved. In this connexion it seems clear that a much fuller investigation of the earliest stages after partial hepatectomy before mitosis begins should be made. It might be possible in this way to find means to investigate the problem of the stimulus in short-term experiments; this would remove a major difficulty in the work. Apart from the question of the stimulus, it is desirable that many of the investigations done on the rat should be repeated on a larger animal—there is, for example, no adequate quantitative information on mitosis in any larger animal. It is likely that new factors may be important in larger animals, in which, for example, the process of regeneration may be modified by the larger proportion of hepatic fibrous tissue.

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THE BILE PIGMENTS

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1 Serum bilirubin and the van den Bergh reaction
2 Kernicterus
3 Biosynthesis of bile pigments
4 Mechanism of bile pigment formation
5 Clinical aspects
6 Conclusion
References

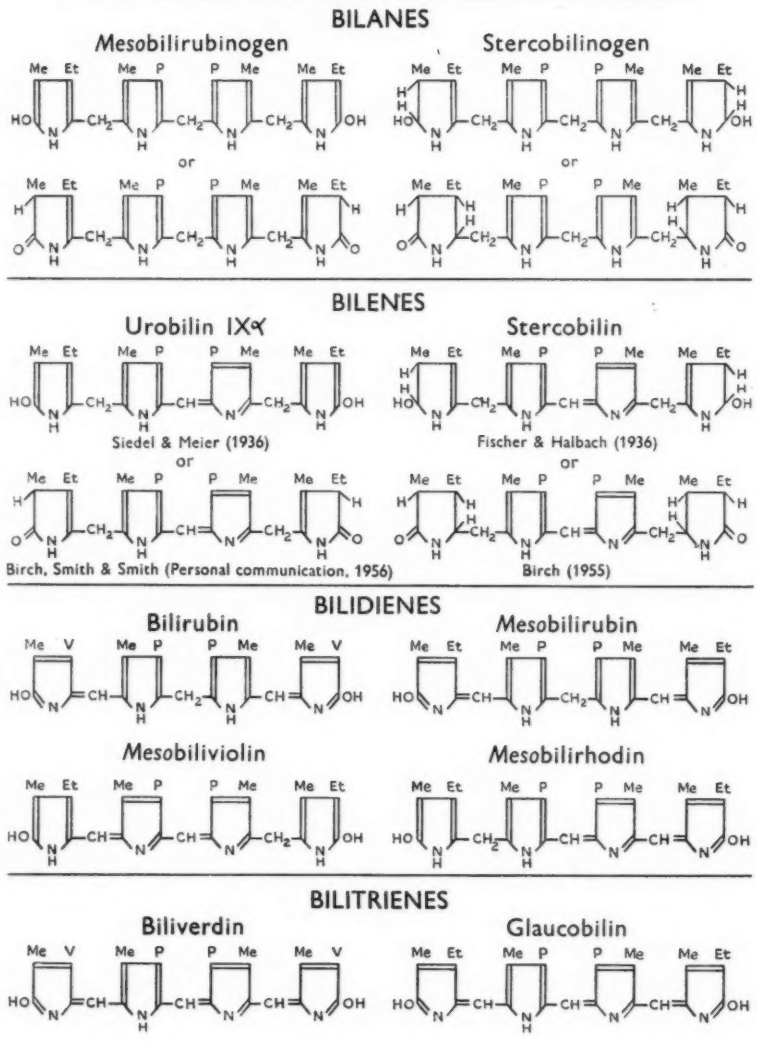
In this article the bile pigments will be regarded as substances the molecules of which consist of a chain with four pyrrole rings, and in keeping with this definition some, such as the precursors of the urobilinoid compounds, will be colourless compounds. Other pigments found in bile, such as porphyrins or porphyrin derivatives from chlorophyll which have a cyclic tetrapyrrole structure, have been excluded. A comprehensive monograph by With (1954) provides a unique and complete account of the biology of the bile pigments.

Figure 1 shows the chemical structures of some of the more important of the naturally occurring bile pigments or those readily derived from them (Fischer & Orth, 1937; Gray, 1953). They may be classified into bilanes, bilenes, bilidienes and bilitrienes, according to the number of double bonds attached to the bridge carbon atoms joining the pyrrole rings. The structures of most of these compounds have been clearly established by synthesis; but this is not so with the urobilinoid pigments, stercobilin and D-urobilin and their colourless precursors, nor with the bilipurpurins and choletelins. Recently, Gray & Nicholson (1957) have isolated methyl ethyl succinimide as a product of chromic-acid oxidation of stercobilin. Although the orientation of the alkyl groups in this compound requires further study, this observation provides the first direct evidence of a reduced pyrrole ring in the stercobilin molecule and supports the lactam structure suggested by Birch (1955) as most consistent with the stability of stercobilin, rather than alternative structures in which pyrrole rings would be destroyed on oxidation. It is very probable that all the bile pigments possess a lactam structure (Birch, Smith & Smith, personal communication, 1956), but in fig. 1 this structure is shown only for the urobilinoid pigments and their precursors.

1. Serum Bilirubin and the van den Bergh Reaction

Since Hijmans van den Bergh & Müller (1916) observed that the diazo reagent without the addition of alcohol reacted with bilirubin in the serum of patients with obstructive jaundice, while alcohol was essential for the reaction to take place with the bilirubin in the sera of normal subjects and of patients with haemolytic jaundice, the mechanism of this difference has been the subject of much research and voluminous literature. The cases of obstructive jaundice described by Hijmans van den Bergh included cases known to him as catarrhal jaundice and regarded by him as obstructive in nature. Such cases are now recognized to be due to a hepatitis with zonal necrosis of liver cells, but it is known that there is no

FIG. 1. STRUCTURAL FORMULAE OF SOME BILE PIGMENTS

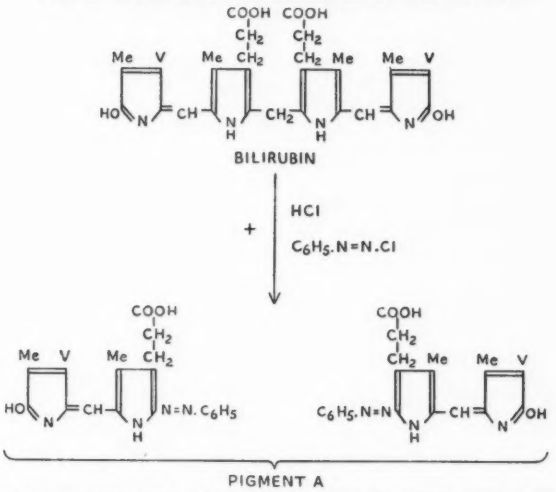


difference in the behaviour of the bilirubin contained in the sera of patients whether they are jaundiced because of obstruction or because of hepatitis. Some workers have postulated that indirect bilirubin, i.e., that giving the diazo reaction only in the presence of alcohol, is bound to plasma protein, while the direct bilirubin which does not require alcohol for the reaction to take place is not bound to plasma protein. Others have believed that direct and indirect bilirubin are separate entities.

Klatskin & Bungards (1956) have recently re-examined by paper electrophoresis the association between bilirubin and the serum proteins. They confirmed earlier work which showed that most, if not all, the bilirubin is bound to albumin, irrespective of its behaviour in the van den Bergh reaction (Martin, 1949; Gray & Kekwick, 1948). Klatskin & Bungards (1956) also showed clearly that serum bilirubin was not a complex of globin as had been suggested by Fiessinger, Gajdos & Polonovski (1941, 1942), and also that fractionation of bilirubin-containing sera with ammonium sulphate did not distinguish between the two forms of pigment as claimed by Najjar & Childs (1953). Klatskin and Bungards concluded that the difference in behaviour of bilirubin in diazotization was probably due to differences in solubility in acid, alcohol presumably increasing the true solubility of indirectly reacting bilirubin under these conditions.

Recently, Billing, Cole & Lathe (1957) have shown that direct reacting bilirubin in sera is not bilirubin itself but a mixture of the mono- and diglucuronides of bilirubin, the monoglucuronide predominating. Cole & Lathe (1953) demonstrated by reverse phase column chromatography from jaundiced serum, two pigments, I and II, each more polar than bilirubin and giving a direct van den Bergh reaction. Subsequently Cole, Lathe & Billing (1954) succeeded in separating the two polar direct reacting compounds and showed that they differed from each other and from bilirubin in respect of their absorption spectra, pigment I having a flat peak at 452 mμ., and often a small peak in the region of 260–280 mμ., and pigment II a plateau at 420–450 mμ.; bilirubin has a peak at 440–445 mμ. Bile and urine from cases of obstructive jaundice were shown in this way to contain pigments I and II as well as small amounts of bilirubin and other diazo-positive pigments. The pigment present in indirectly reacting sera has been readily crystallized (Hijmans van den Bergh, 1918) and was shown by Lathe and his colleagues to be bilirubin itself. The instability of the directly reacting pigments has proved an insuperable obstacle to their isolation in the pure state. Billing *et al.* (1957) have therefore extensively purified by partition chromatography, and by counter-current distribution, pigment B, the compound obtained by the action of benzene diazonium chloride on the directly reacting pigment in bile. Pigment B, which differed from the corresponding pigment A prepared from bilirubin, was found on alkaline hydrolysis or by the action of β-glucuronidase to give pigment A and glucuronic acid. Although the preparation was only about 87% pure, analysis showed that the molar proportion of glucuronic acid to pigment A was one. Since one molecule of bilirubin yields two molecules of the isomeric components of pigment A (Overbeek, Vink & Deenstra, 1955) (fig. 2), pigment II was considered to be the diglucuronide of bilirubin (fig. 3), a view supported by the observation that alkaline hydrolysis converted pigment II into bilirubin. This was in agreement with the observation by Talafant (1956) that β-glucuronidase

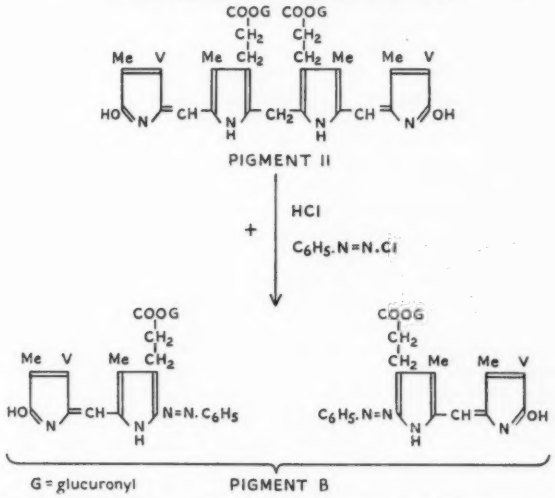
FIG. 2. THE DIAZO REACTION OF BILIRUBIN



Note. Pigment A is a mixture of two isomeric dipyrryl compounds.

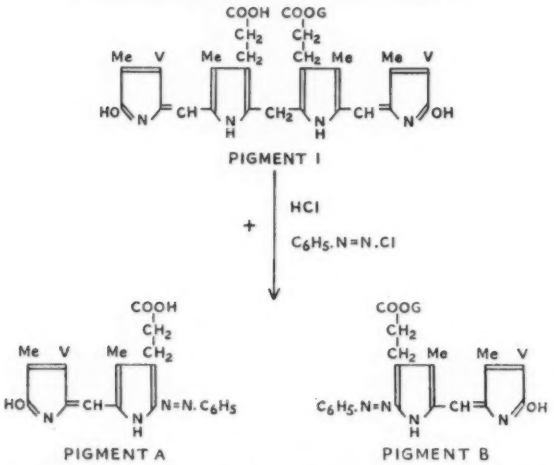
converted into bilirubin directly reacting pigment separated by electrophoresis from bile. Subsequent analyses of directly reacting sera and of bile showed that increased amounts of glucuronic acid were present. Insufficient amount was present in bile to account for all the directly reacting pigment as bilirubin diglucuronide. Billing (1954) found that pigment I yields both pigment A and B in the diazo reaction (fig. 4); this suggests that pigment I was bilirubin monoglucuronide. Meanwhile Jirsa, Večerek & Ledvina (1956) have synthesized compounds in which one or both carboxyl groups of bilirubin are combined with taurine. The resulting di- and monotaurobilirubin behaved very similarly to the glucuronide of bilirubin

FIG. 3. THE DIAZO REACTION OF BILIRUBIN DIGLUCURONIDE (PIGMENT II)



Note. Pigment B is a mixture of the glucuronides of two isomeric dipyrryl compounds.

FIG. 4. THE DIAZO REACTION OF BILIRUBIN MONOGLUCURONIDE (PIGMENT I)



Because of the unsymmetrical arrangement of the β side-chains in the bile pigments, pigment I can occur in two isomeric forms differing in the particular carboxyl group to which the glucuronyl radical is attached. Pigments A and B in this reaction can therefore show the isomerism indicated in figs. 2 and 3.

described by Lathe and it seems not impossible that some of the minor constituents in bile giving the direct van den Bergh reaction may prove to be related to such compounds.

Similar findings have been reported by Schmid (1956) who has also compared the properties of the purified azo compound from bilirubin with that obtained from synthetic neoxanthobilirubic and *iso*neoxanthobilirubic acids. He has hydrolysed with acid and with β-glucuronidase the purified azo pigment from bile and from the urine of jaundiced patients and obtained azo pigment with the same chromatographic properties as that obtained from bilirubin. Japanese workers have also been active in this field (Shimada, 1954; Sakamoto, 1956).

This work has greatly clarified a distinction, hitherto wholly empirical, but of considerable diagnostic significance in clinical medicine. Like other physiological substances such as the steroids, bilirubin is conjugated presumably by the liver with glucuronic acid to form water-soluble compounds differing in physical properties from the water-insoluble lipophilic bilirubin. Bilirubin itself is present in the sera from patients with uncomplicated haemolytic (retention) jaundice. Bilirubin is insoluble in aqueous media at physiological pH and in the acid reaction of the diazo reaction mixture, and is presumably held in solution in serum only by virtue of its linkage with the serum proteins. Lathe (1956) implies that the presence of alcohol in the diazo reaction merely provides a suitable solvent to enable the bilirubin to pass into true solution and allow the diazo reaction to proceed. This view, as we have seen, was also held by Klatskin and Bungards. The absence of bilirubin in true solution in such sera means that the pigment does not pass by glomerular filtration into the urine. The urine in such cases does not contain bilirubin.

In regurgitation jaundice (whether due to obstruction or to liver damage), the pigment circulating in the blood is mainly conjugated bilirubin, the greater proportion of which is bilirubin monoglucuronide with smaller amounts of the diglucuronide and perhaps of other conjugated forms of bilirubin as well. Although these conjugated forms of bilirubin are bound to the same proteins as the bilirubin in normal sera and those from subjects with haemolytic jaundice, the water solubility of the conjugates of bilirubin permits their excretion in the urine. There is no evidence available to indicate whether this is by glomerular filtration or by tubular excretion or both. Since there is binding by the plasma proteins, ultrafiltration through the glomeruli seems unlikely, a view supported by the absence of pigment, even in severe obstructive jaundice, in the cerebrospinal fluid, in the formation of which ultrafiltration also presumably plays a part.

2. Kernicterus

The affinity of bilirubin for lipids may lead to deposition of the pigment in the brain tissue when the amount in the serum is in great excess (Claireaux, Cole & Lathe, 1953). This does not usually occur in most cases of haemolytic jaundice because the serum bilirubin seldom exceeds about 10 mg./100 ml. However, in haemolytic disease of the newborn and of premature infants, a serum bilirubin of 30 mg./100 ml. or more is not uncommon, and then appreciable quantities of the pigment are deposited in the brain, particularly in the basal ganglia, resulting in the so-called " kernicterus ". Billing, Cole & Lathe (1954) have emphasized that the severe jaundice occurring in haemolytic disease of the new-born and the premature infant is greater than can be explained by the increased rate of haemoglobin breakdown and that the functional capacity of the liver to excrete bilirubin must be of the order of 1–2% of the normal adult capacity.

A possible effect of free bilirubin on tissue metabolism has been shown by Zetterström & Ernster (1956). They showed that, in vitro, uncoupling of oxidative phosphorylation in isolated mitochondria is brought about by bilirubin in concentrations which, in the blood of infants, are known to be associated with kernicterus.

3. Biosynthesis of Bile Pigments

Of the bile pigments, stercobilin is the one end-product of haemoglobin metabolism and perhaps of other haem protein metabolism which is readily isolated in the pure state to permit isotope studies in the human. After ^{15}N-labelled glycine has been fed to a normal person, the faecal stercobilin ^{15}N time-curve shows two peaks, the first immediately after glycine feeding and the second with a maximum about the 130th day when the declining haem curve shows that the labelled red cells are breaking down. The latter peak normally accounts for about 80% of the labelled stercobilin. The two peaks clearly indicate that stercobilin is formed not only from cells at the end of a normal life-span but also by a more rapid mechanism, possibly from haem synthesized in excess of globin and therefore not required for the formation of haemoglobin. In pernicious anaemia and in congenital porphyria, the stercobilin ^{15}N time-curve is very different; the first peak is greatly exaggerated and the second peak barely noticeable, so that the breakdown of red cells at the end of their normal life-span in these conditions is responsible for only about 15% of the excreted bile pigment, which is formed from glycine by the rapid mechanism in these two conditions.

In normal people as well as in such patients there was always a small, but significant, labelling between the two peaks which at first was thought to indicate bile pigment formation from non-haemoglobin haems, but Berlin, Hewitt & Lotz (1954) of Berkeley, California, have recently shown that the persistence of labelling at this time is because the glycine pools of the body remain labelled for longer than at first seemed probable. They have also explained the last part of the ^{15}N haem time-curve, which has interested workers for some time. Investigations by the agglutination technique have shown that practically no cells survive beyond the 130th day, yet the experimental data from isotope experiments show that the ^{15}N content of the haemoglobin haem is still about 10–15% of its maximal value 150 days after feeding, and a significant amount is still present after 250 days. Neuberger (1951) suggested that this might indicate that a proportion of the haem or of the haemoglobin of decaying cells might be re-utilized in the production of new cells even in normal man. Berlin *et al.* (1954) have studied the radioactivity of urinary hippurate at various times after the administration of ^{14}C-labelled glycine. The rate of decrease of the specific activity of this hippuric acid could be described in terms of two exponential components having average half-times of 7 and 75 days. The existence of this second exponential component showed clearly the error of the earlier assumption that the glycine contained a negligible excess of isotope 20 days after the glycine feeding. They concluded that the last portion of the curve of specific activity of haemoglobin haem could be due in large measure, if not entirely, to the continued formation of labelled red cells derived from the persisting labelled glycine rather than to a re-utilization of haem or haemoglobin.

4. Mechanism of Bile Pigment Formation

The chemical mechanism of breakdown of haemoglobin to bile pigments in vivo remains obscure. Although the brilliant investigations of Lemberg (see Lemberg & Legge, 1949) in this field have been confirmed and extended in Japan (Imai, 1954), Australia (Lemberg, 1956), Scandinavia (Sjöstrand, 1952) and Great Britain (Kench, 1954), little direct evidence has accrued to indicate the proportion of total haemoglobin broken down in vivo via choleglobin. The precise pathway of breakdown of haemoglobin and of other haem proteins to bile pigments in vivo will be difficult to establish. At present, the evidence is circumstantial, and the presumed intermediate products—although recognizable by their spectroscopic properties—have proved resistant to isolation in the pure condition. Isotope experiments will be impossible until these compounds can be obtained pure.

London, Yamasaki & Sabella (1951) have found labelled stercobilin in the faeces after ^{14}C-labelled protoporphyrin administration, in extension of London's earlier finding (1950) that intravenously administered ^{15}N-labelled haematin was converted in the body to labelled stercobilin. Such experiments provide no evidence of the normal pathway, since these compounds may be converted directly to bile pigment or iron may be introduced into the porphyrin molecule, the resulting haematin may combine with a protein, and the breakdown may then follow the pathway described by Lemberg.

5. Clinical Aspects

The diazo reaction, especially by the method of Malloy & Evelyn (1937), remains the most satisfactory for the quantitative estimation of serum bilirubin. Until the separate estimations of bilirubin and the two forms of conjugated bilirubin by the method of Billing (1955) have been shown to be of clinical importance, it seems unlikely that this technique (which is too time-consuming to be readily acceptable for routine use) will supplant the easier estimations of " 1-minute or 30-minute direct bilirubin ", based on the Malloy and Evelyn method. These estimations are useful in the diagnosis of haemolytic and other forms of retention jaundice, for example, the familial retention jaundice of Dameshek & Singer (1941), but are of no value in the differential diagnosis of obstructive jaundice from hepatogenous jaundice (Gray, 1947).

The importance of qualitative tests in the investigation of jaundice is clear from Table I. The detection of bilirubin in urine, using nitric acid or a solution of iodine as oxidizing agent, has been superseded in Great Britain at any rate by the Fouchet test after adsorption on a barium sulphate precipitate (Harrison, 1939) or on a filter paper strip impregnated with barium sulphate (Watson & Hawkinson, 1946). The development of a stable solid diazo reagent by *p*-nitrobenzene diazonium *p*-toluene sulphonate has been made the basis of a convenient tablet test requiring no liquid reagents. Tallack & Sherlock (1954) have shown that this is as sensitive as the Fouchet test, and its convenience and simplicity suggest that it may become widely used. Although Bryant & Flynn (1955) reported that it was not quite so sensitive, it appears that these workers introduced a modification into the method. The Ehrlich aldehyde test for urobilinogen and the Schlesinger reaction for urobilin and urobilinogen have not been improved. Theoretically, the former test carried out on fresh urine should suffice, but the Schlesinger test is of value in confirming that a positive reaction in the aldehyde test really is due to " urobilinogen ". In this way, urobilinogen is readily distinguishable from porphobilinogen (Gray & Rimington, 1947), or the tests may indicate the need for further investigation of Ehrlich-reacting chromogens other than " urobilinogen " by methods based on that recently developed by Dalgliesh (1955).

TABLE I. BILE PIGMENTS IN URINE

	Bilirubin	Urobilinogen and/or urobilin
Normal	0	trace
Haemolytic jaundice	0	trace or +
Obstructive jaundice	+	0
Liver damage (mild)	+	+
Liver damage (severe)	+	0
Obstructive jaundice and infection	+	+

The estimation of " faecal urobilinogen " (Watson, 1938; Maclagan, 1946; Gray, 1957) is of value in the diagnosis of obstructive jaundice due to a malignant obstruction, and in that of haemolytic anaemia. In the former condition, biliary obstruction is complete, and " faecal urobilinogen " is persistently less than 5 mg. per day. In haemolytic anaemia or jaundice, the daily excretion is often greatly in excess of 250 mg. per day. Such measurements cannot provide an accurate assessment of haemolysis, not only because of the demonstration with isotopes that a proportion, often substantial, of the faecal stercobilin is derived from a source other

than red cells at the end of their life-span (for example, in congenital porphyria—London, West, Shemin & Rittenberg, 1950; Gray, Neuberger & Sneath, 1950), but also because of conversion of bile pigment in the gut into substances not estimated by the usual techniques for measuring stercobilin. Nevertheless, if these limitations are borne in mind, estimations of " faecal urobilinogen ", especially when the results are interpreted in relation to the amount of circulating haemoglobin, may be of value in supporting haematological evidence of haemolysis.

Lemberg, Lockwood & Wyndham (1938) suggested that in liver damage the proportion of urobilin IXα to stercobilin in urine " urobilin " was greater than normal, and later Legge (1949) showed a similar effect on faecal urobilin when rats were treated with sulphonamides. Investigations in my Department have shown that the method of Legge is not entirely satisfactory for the differential estimation of these two pigments and that the significance of the partition of the various urobilinoid pigments in faeces and urine is still not clear. Shigei (1954) has investigated the formation of urobilinogen from bilirubin by faecal flora and has been unable to confirm completely the observations of Baumgärtel (1950) on the role of cysteine-cystine in the reduction of bilirubin in the intestine. This would therefore cast doubt on Baumgärtel's hypothesis of an hepatic origin of *meso*bilirubinogen, the precursor of urobilin IXα, a view which at one time appeared to support the possibility that investigation of the urobilinoid pigments in urine and faeces might be of diagnostic value. The clinical significance of D-urobilin, the dextrorotatory urobilinoid isolated from faeces after antibiotic therapy (Lowry, Ziegler & Watson, 1952) remains obscure.

6. Conclusion

It is clear that the most important advance made in this field during the last ten years has been the elucidation of the mechanism of the van den Bergh reaction and the observation that bilirubin is excreted by the liver in conjugated form. It seems probable that a number of related problems may become clarified in the near future. One may thus expect work on conjugated forms of bilirubin other than the glucuronides, perhaps on conjugated forms of urobilinoid pigments in urine and faeces, as well as investigation concerning the precise fate of reabsorbed urobilinogen. When methods of investigation and analysis are improved it is not impossible that isomers of bilirubin may be detected in nature. Perhaps some advances will be made in elucidating the pathway of breakdown of haemoglobin to bile pigment, but at present it is difficult to see the direction which this work is likely to take.

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RECENT ADVANCES IN RADIOLOGY OF THE BILIARY AND PORTAL SYSTEMS

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1 Cholangiography and cholecystography
2 Portal venography
a Methods
b Complications
c Clinical value
References

Recent years have seen major advances in the radiology of the biliary and portal systems. The more important of these may be discussed under the headings of (i) cholangiography and cholecystography and (ii) portal venography.

1. Cholangiography and Cholecystography

Operative cholangiography has for a number of years found an effective place in the control of operations on the gall-bladder and bile ducts. Similarly post-operative cholangiography is considered by many surgeons to be of great value in patients who have undergone biliary surgery. It is certain that the surgeon who routinely performs such cholangiograms is far less likely to leave stones behind than is the surgeon who omits the procedure. Norman (1951) has published a monograph on the subject of operative cholangiography and he emphasizes that to obtain the best results the procedure requires the closest co-operation at the time of operation between surgeon, radiologist and anaesthetist. A first-class radiological technique, despite the operative conditions, is essential to the procedure if significant results are to be obtained. The choice of contrast medium is also important. Iodized oils, which have been widely used in the past, have a tendency to fragment and to include air bubbles, thus giving rise to confusion and misinterpretation of signs. A water-soluble medium such as 35% diodone enables much more accurate results to be obtained.

The most spectacular advance in the field of cholangiography, however, was the introduction of a new intravenous cholangiographic medium in 1953. In this way the procedure of visualizing the bile ducts has extended from the patient in the operating theatre, or with a post-operative drainage tube, to the ambulant out-patient with intact abdomen.

Effective cholecystography, as distinct from cholangiography, began in 1924 when Graham & Cole published their pioneer work. Since that date cholecystography has usually been carried out by oral administration of the drug to be excreted in the bile. Oral cholecystography, particularly with the newer media introduced in recent years, gives very adequate results in a high proportion of cases. There are, however, certain defects inherent in the oral method, which in many cases result in equivocal radiological findings. This is because the dose absorbed by the patient through the gut cannot be predicted in advance or controlled, and may be negligible. In addition, toxic effects occur in a high proportion of cases with all the oral media and, even with the most innocuous of them, rather more than one-third of the patients suffer reactions such as nausea, diarrhoea or vomiting. This applies even to Telepaque, the most recent and best of the oral media. It has long been felt that a safe intravenous method of cholecystography would offer certain advantages. Thus the exact dose received by the patient would be controllable and the amount of the drug reaching the blood stream would be guaranteed. Attempts at intravenous cholecystography in the past have been discouraged, however, because of the toxicity of the media until now available. However, in 1953 reports appeared of a new intravenous medium named Biligrafin and produced by Messrs Schering in Germany. Several German workers claimed remarkable results with this new medium (Frommhold, 1953a, 1953b; Hornykiewytsch & Stender, 1953; Gaebal & Teschendorf, 1953).

A full-scale clinical trial was begun at St Mary's Hospital, London, in 1953 by Sutton & Tillett. This was reported in the *British Journal of Radiology* in October 1954, and their findings fully confirmed the preliminary claims of the German workers. These claims have also been amply endorsed from many other centres.

In summary, the findings with regard to Biligrafin are as follows:

i. It is virtually non-toxic, and though given by the intravenous route reactions are negligible.

ii. Excretion by the liver is rapid and in a concentrated form. Thus not only is there an adequate cholecystogram but cholangiography is possible in the majority of cases.

iii. Cholangiography is possible after the gall-bladder has been removed.

It is clear therefore that the introduction of Biligrafin represents a major advance in the radiology of the biliary tract, particularly as it makes non-operative cholangiography possible.

The indications for the use of Biligrafin and its limitations are now fairly well established. It is undoubtedly the best method of obtaining an anatomical visualization of the biliary tract. However, it involves a rather more cumbersome technique than does simple oral cholecystography, and for this reason the latter is still widely practised. It should also be pointed out that recent refinements in oral cholecystography have made possible visualization of the common bile duct in a fair proportion of cases, though duct visualization cannot be as consistently achieved as by the intravenous method. In the cholecystectomized patient intravenous cholangiography is the only pre-operative method of demonstrating the bile ducts and the possible presence of stone. It is thus of considerable value in the investigation of the patient with recurrent symptoms after cholecystectomy. Refinements in the technique of intravenous cholecystography have been suggested by Aldridge (1955), who administers pethidine at the time of intravenous injection of the Biligrafin in order to cause closure of the sphincter of Oddi. He also administers a fatty meal shortly before the injection in order to empty

the biliary tract of residual bile which might dilute the Biligrafin.

To sum up: the judicious use of the newer techniques of cholecystography and cholangiography should permit an accurate anatomical diagnosis to be made in the majority of cases of biliary disease. The value of intravenous cholecystangiography in the functional assessment of the biliary tract is still under investigation.

One drawback to the use of these methods is that they are less effective in the jaundiced patient. Sutton and Tillett obtained no visualization of the biliary tract in 15 jaundiced or recently jaundiced patients. On the other hand, Aldridge (1955) claims that some success can be achieved even in these cases.

2. Portal Venography

a. Methods

The procedure of portal venography by percutaneous injection of the spleen was originated by Abeatici & Campi (1951) who performed the procedure in dogs. The method was then successfully applied in man (Leger, 1951; Boulvin, Chevalier, Gallus & Nagel, 1951; Campi & Abeatici, 1952). The method has gained rapid acceptance and is now practised routinely at many centres. In Great Britain, Atkinson, Barnett, Sherlock & Steiner (1955) have reviewed a series of 40 cases.

Whilst the percutaneous trans-splenic technique is the method most widely used, some prefer to perform portal venography at laparotomy by injection of a mesenteric vein (Du Boulay & Green, 1954).

At St Mary's Hospital we have practised mainly the trans-splenic method, though a few operative venograms have been done at laparotomy (Sutton, 1956).

The investigation of percutaneous splenic venography is usually performed under local anaesthesia after preparation by a basal narcotic. The patient lies supine on the *x*-ray table, and the spleen is punctured in the mid-axillary line either below the costal margin or between the lower ribs. The procedure is not difficult to perform, as most of the patients investigated have enlarged spleens. However, a spleen of normal or small size may prove difficult to puncture. We use needles of 19 or 18 standard wire gauge. Most of the cases investigated suffer from portal hypertension, and puncture of the spleen is immediately followed by a fairly rapid dripping of blood through the needle into the plastic connecting tube which links the needle with the injection syringe. Atkinson & Sherlock (1954) practise measuring the intrasplenic pressure before proceeding to venography.

We perform the procedure as rapidly as possible and the needle is rarely left in the spleen for more than two or three minutes including the time for injection of contrast medium and exposure of *x*-ray films. It is removed immediately the films have been taken. During the procedure the patient is instructed to practise only shallow respirations in order to avoid undue trauma to the spleen. We inject from 20 to 50 ml. of the contrast medium. This can be either diodone (50% or 70%) or a tri-iodated compound such as Diaginol (70%). Rapid serial films are taken, commencing half-way through the injection which is made as rapidly as possible.

For the operative technique of portal venography a mesenteric vein is usually isolated at operation, and the contrast medium injected into it (Plate III, fig. G). The spleen can also be directly injected at operation.

b. Complications

Du Boulay and Green claim that the danger of splenic rupture makes the investigation at operation the method of choice, but most workers consider that there is little such risk associated with the trans-splenic method. It is obvious, however, that the patient should be carefully watched for some days after the investigation for signs of splenic rupture if operation is to be delayed. In many cases the investigation is usually undertaken as a pre-operative measure. No complications have been encountered in a series of 30 cases examined at St Mary's Hospital in the last three years.

As with all injections containing iodine, the possibility of sensitivity to the contrast medium exists, but this risk is generally considered to be negligible, and no greater than that associated with intravenous pyelography.

Cases have been recorded where accidental injection has been made into the peritoneum, into the ascending colon, and even into the lung, but these accidents appear to have had no serious consequences.

c. Clinical Value

The main use of portal venography is in investigating the cause of portal hypertension, and in differentiating between intra-hepatic and extra-hepatic portal obstruction. Portal venography will often provide invaluable information in this type of case. The size and patency of the portal vein can be demonstrated and the feasibility of porta-caval anastomosis assessed. Similarly the size and shape of the splenic vein can be demonstrated for a proposed spleno-renal anastomosis where the portal vein is obstructed, or a splenic vein thrombosis may be demonstrated. Following operation the functioning of a porta-caval anastomosis may be difficult to assess clinically, and a splenic venogram done after the operation will demonstrate the patency or otherwise of the surgical anastomosis.

The following are some illustrative cases which have been investigated at St Mary's Hospital:

Case 1. A child with a clinical diagnosis of Banti's syndrome: percutaneous trans-splenic portal venography was performed (Plate II, fig. A). This showed that the child had a large splenic vein but that the portal vein was replaced by a number of small vessels representing a so-called " cavernoma " of the portal vein. This was probably secondary to a portal thrombosis. The patient was transferred to Bristol where a spleno-renal shunt was performed by Mr R. Milnes Walker.

Case 2. This patient was admitted to hospital with a history of two severe haematemeses, probably due to cirrhosis of the liver. The trans-splenic portal venogram demonstrated large splenic and portal veins and showed that a porta-caval anastomosis was feasible (Plate II, fig. B). This was later performed. A post-operative splenic venogram was then done (Plate II, fig. C), and the portal vein was visible emptying into the inferior vena cava.

Case 3. A young woman aged 24 was admitted with a history of repeated haematemeses. A splenic venogram showed a patent splenic and portal vein (Plate II, fig. D). A porta-caval shunt was performed, but the clinical result was poor and there was clinical doubt as to whether the anastomosis was functioning. A post-operative venogram (Plate III, fig. E) showed that the anastomosis had been unsuccessful and that the portal tract was now filling from enlarged periportal veins.

Case 4. A girl aged 15 with a history suggestive of cirrhosis of the liver and episodes of haematemesis and haematuria: splenic venography showed most of the contrast medium draining via an enormous mesenteric vein to the hemiazygos and other retroperitoneal veins (Plate III, fig. F). Only a trace of contrast medium was seen in the distal part of the splenic vein and in the portal vein. The appearances suggested a partial

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(FIGS. A—H)

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FIG. A. Film from a splenic venogram series showing the contrast medium entering the liver through numerous tortuous small vessels replacing the portal vein. The contrast medium has also emptied from a normally patent splenic vein but is still present in gastric and oesophageal varices and in the inferior mesenteric vein. (Case 1: male, age 10.)

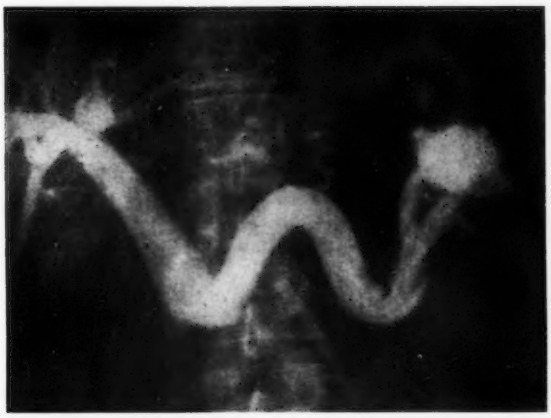

FIG. B. Trans-splenic venogram (case 2: female, age 43) showing normally patent splenic and portal veins

FIG. C. Post-operative splenic venogram (case 2) showing portal vein emptying into the inferior vena cava

FIG. D. Pre-operative venogram (case 3: female, age 24) showing normally patent splenic and portal veins

FIG. E

FIG. F

FIG. G

FIG. E. Post-operative venogram (case 3) showing failure of a porta-caval shunt operation, with contrast medium still reaching the liver from new splenic portal anastomoses

FIG. F. Splenic venogram (case 4: female, age 15) showing most of the contrast medium passing from the spleen into a huge single mesenteric drainage vein. The splenic and portal veins are faintly outlined

FIG. G. Operative portal venogram by injection of a mesenteric vein (case 4) showing normally patent superior mesenteric and portal veins and gastric varices

FIG. H (a, b). Splenic venogram (serial films) (case 5: male, age 42) showing single large anastomotic vessel shunting blood from the portal vein

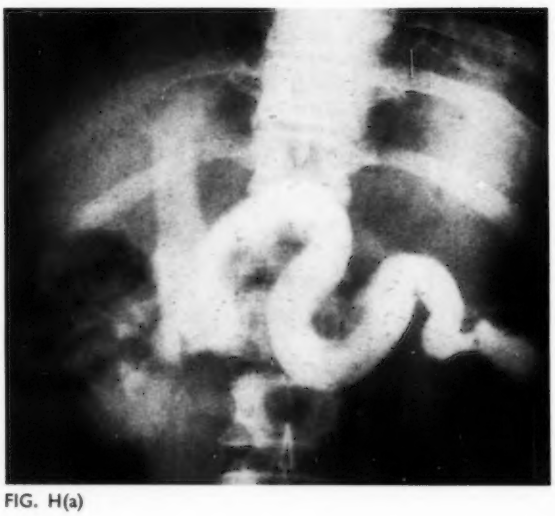

FIG. H(a)

FIG. H(b)

thrombosis in these vessels. Such an appearance we have found can be most misleading, and an operative portal venogram was therefore done by injection of a mesenteric vein (Plate III, fig. G). This demonstrated a normally patent superior mesenteric and portal vein, together with gastric varices, and proved conclusively that the portal hypertension was due to an intra-hepatic obstruction.

Case 5. The patient was a man aged 42 with a history of haematemesis and melaena and a clinical diagnosis of hepatic cirrhosis. Splenic venography demonstrated that most of the contrast medium was passing through the splenic and portal veins and then through an enormous single anastomotic vein, probably mesenteric, to reach the iliac veins. (See Plate III, figs. Ha and Hb.)

These illustrative cases demonstrate the wide range of anatomical changes which may be present in patients with portal hypertension. The value of radiological investigation in the assessment of the case and in the control of surgical treatment is clearly evident. Plate III, fig. F, demonstrates a possible pitfall in the radiological diagnosis, in that failure to show the portal vein clearly does not necessarily mean that it is obstructed or occluded, particularly where most of the splenic blood is being drained by a single major anastomotic vessel.

In some cases a similar large anastomotic vessel may drain the blood from the portal vein itself and in this case it may be regarded as a natural shunt. If operation is decided upon, every effort must be made to preserve this important naturally developed anastomosis.

The wide-spread use of trans-splenic portal venography has demonstrated the existence in cases of portal hypertension of numerous collateral channels besides those generally recognized. An excellent survey of the anastomoses in cases of portal hypertension, based on post-mortem radiological studies, has been published by Doehner, Ruzicka, Rousselot & Hoffman (1956).

It has been claimed that fairly typical hepatic vascular patterns are seen in the liver in cases of intra-hepatic and supra-hepatic portal obstruction, which may help in differentiating the two conditions and in the diagnosis of cirrhosis (Basu & Das, 1956). Whilst this may be so, the variations in the radiological appearances of the intra-hepatic venogram require further detailed study and, until more material has been evaluated, such claims must be treated with reserve.

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EXPERIMENTAL STUDIES ON TOXIC LIVER INJURY

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1 Classification of toxic liver injury
2 Problems of toxic liver injury
3 Functional studies in toxic hepatic necrosis
4 Specificity of action and the metabolism of toxic agents
5 The biochemical mechanism of action of liver poisons
6 Conclusions
References

" Les poisons peuvent être employés comme agents de destruction de la vie ou comme moyens de guérison des maladies; mais, outre ces deux usages bien connus de tout le monde, il en est un troisième qui intéresse particulièrement le physiologiste. Pour lui, le poison devient un instrument qui dissocie et analyse les phénomènes les plus délicats de la machine vivante, et, en étudiant attentivement le mécanisme de la mort dans les divers empoisonnements, il s'instruit par voie indirecte sur le mécanisme physiologique de la vie."

Claude Bernard (1878)

As part of the increased attention being paid to diseases of the liver there has been a revival of interest in toxic liver injury. The liver is an organ where toxic injury is sufficiently frequent to form a clinical problem and, with the growing use of chemicals in every type of industry, a better understanding of its causes and mechanism is necessary for the safety of all. This alone would be sufficient reason for the experimental study of toxic liver damage but the problems can also be approached in another way—in the spirit described above by Claude Bernard. The experimental investigation of toxic liver injury from this point of view may well yield results applicable to liver disease in general. The enormous growth in knowledge of the physiology and biochemistry of the liver in recent years should help the elucidation of its disordered function in disease. The aim of this review is to show the progress being made in the application of physiological and biochemical methods to these problems.

1. Classification of Toxic Liver Injury

Liver injury is produced by a host of different substances and, since similar lesions may be produced by very different compounds, it is impossible to use chemical structure as a means of classification. This is most usefully based on the anatomy of the lesion so that hepatotoxic agents have been classified as those causing " centrilobular ", " mid-zonal " or " periportal " damage according to the initial site of the lesion in the classical liver lobule. Recent work on the microanatomy of the liver by Elias (1955) and Rappaport, Borowy, Lougheed & Lotto (1954) is leading to a fuller appreciation of the relationship between the liver cells and their blood supply, and, when it is combined with histochemical studies (see, for example, Deane, 1944; Chiquoine, 1953), it should result in a better understanding of the distribution of the lesion in the liver. Ultimately classification may be in physicochemical terms but, until that is possible, it is useful to retain the classical terminology. Table I, which is not intended to be exhaustive, shows how different chemicals may be grouped in this way. Compounds such as α-naphthyl *iso*thiocyanate (Lopez & Mazzanti, 1955) and the dialkyl tin compounds (Magee & Barnes, 1957), which seem primarily to affect the biliary system, are not included. Known hepatic carcinogens are indicated in the table but we have not attempted to separate them further, since: " In general, it may be assumed that all necrogenic hepatoxic agents are potential carcinogens for the liver . . ." (György, 1954). This has been discussed by Magee & Barnes (1956). The production of liver tumours by toxic agents creates special problems which have been widely reviewed (Orr, 1947; Wilson, 1955) and cannot be discussed here. Since hepatic carcinogens such as *p*-dimethylaminoazobenzene (Orr & Price, 1948) and the pyrrolizidine (Senecio) alkaloids (Cook, Duffy & Schoental, 1950) also produce acute hepatic necrosis, further investigation of their acute effects might yield valuable information on both scores. In this connexion the chronic lesions produced by single doses of the latter compounds (Schoental & Magee, 1957) are of particular interest. As the scope of this review must necessarily be limited, consideration of the influence of diet and hormones on toxic liver injury has also been excluded (cf. Drill, 1952; Miner, 1954; Hartroft, 1956).

2. Problems of Toxic Liver Injury

Hepatic necrosis is the morphological result of an alteration in cellular metabolism, and the study of its production by toxic agents is largely an attempt to answer the following questions:

i. Which cellular processes are so disrupted by the toxic agents that the cells die?

TABLE I. TOXIC AGENTS CAPABLE OF CAUSING ACUTE ZONAL NECROSIS WHEN GIVEN IN A SINGLE DOSE. (Those indicated with an asterisk will also produce primary hepatic tumours when given in small repeated doses.)

Area of liver lobule affected	Compound
Centrilobular	Carbon tetrachloride (CCl_4)* Chloroform ($CHCl_3$)* Bromobenzene Dimethylnitrosamine* Sodium fluoroacetate *p*-Dimethylaminoazobenzene* Pyrrolizidine (Senecio) alkaloids* Thioacetamide* Tannic acid* Trinitrotoluene
Mid-zonal	Beryllium
Periportal	Allyl formate Phosphorus Endotoxin of *Proteus vulgaris*

ii. Why is the damage often confined to those cells with a particular anatomical distribution within the liver?
iii. Why is the liver predominantly or, in some cases, specifically affected by these compounds?

The complexity of the problems raised by these questions is indicated by the variety of the chemical compounds producing centrilobular necrosis alone and by the cytological differences in the lesions produced by different agents at the same site. The final disruption of the cells may be due to a common series of chemical reactions; but those reactions may be set in motion in many different ways, depending on the particular toxic agent employed.

3. Functional Studies in Toxic Hepatic Necrosis

The metabolic changes in the liver leading to necrosis might be due to the action of the toxic agent on the affected cells or they might be secondary to circulatory alterations in the liver. Elsewhere in the body, necrosis is usually the result of ischaemia, so it is natural to consider this possibility in the liver especially as centrilobular necrosis occurs in circulatory failure. The view that vascular changes play an important part in the production of toxic hepatic necrosis, particularly the centrilobular type, was developed by Himsworth (1947) and may be put as follows: necrosis, such as that due to CCl_4, is centrilobular because the sinusoids are narrowed by swelling of the parenchymal cells; this makes the central cells ischaemic and finally necrotic, whereas after a compound like allyl formate these cells are protected by dilatation of the sinusoids, and the poison exerts a direct toxic effect on the periportal cells. This idea was widely accepted (Andrews & Maegraith, 1948; Baxter, 1948; Williams, 1948; Drill, 1952; Elias, 1955; Klatskin, 1956) and was extended to explain the massive hepatic necrosis and diffuse fibrosis produced in rats by dietary means (Himsworth, 1947; Glynn, Himsworth & Lindan, 1948).

Evidence for this theory was obtained by studying the intra-hepatic distribution of Indian ink injected into the portal system of the anaesthetized rat at laparotomy (Glynn & Himsworth, 1948; Andrews & Maegraith, 1948; Baxter, 1948; Glynn *et al.* 1948). With this method restriction of the intralobular circulation was described as early as 4 hr. after the subcutaneous injection of CCl_4. However, Daniel, Prichard & Reynell (1952) and Cheng (personal communication) obtained variable results with it, and radiographic studies failed to reveal any alteration in the vascular pattern of the liver or rate of portal blood flow in acute CCl_4 poisoning (Daniel *et al.* 1952). On the other hand intra-hepatic vasoconstriction had been observed in the edge of the exposed liver, under certain conditions, by the quartz-rod transillumination technique after both $CHCl_3$ and CCl_4 (Loeffler & Nordmann, 1925; Wakim & Mann, 1942), although Seneviratne (1949) reported dilatation of the sinusoids in the early stages and could only find vasoconstriction after 24 hr. when necrosis had occurred.

Through the work of Grayson (1952) in developing Gibbs' (1933) heated thermocouple it has become possible to assess blood flow and measure temperature in the liver of the unanaesthetized rat. This method has its own imperfections but in pathological investigations these are probably far outweighed by its advantage in giving information on the conscious animal. When the liver is injured by a substance such as $CHCl_3$ the damage is very uniformly distributed (Calder, 1942) so that, if the centrilobular necrosis were due to ischaemia, the circulatory disturbance should be gross and well within the sensitivity of this method—especially as Baker (1956) has shown that ischaemia for 30–40 min. is necessary to produce necrosis in the rat liver. A number of hepatotoxic agents have now been studied with this technique and it has been found that centrilobular necrosis can be produced in the rat without any reduction in hepatic blood flow. Indeed, with CCl_4 and dimethylnitrosamine the blood flow around the heated thermocouple was actually increased while the liver was undergoing necrosis (Stoner, 1956). The divergence between these results and those obtained by the dye injection and transillumination methods requires explanation; this is probably to be found in the greatly increased sensitivity of the circulation in the damaged liver to the depressant effect of the anaesthesia and laparotomy necessary to expose the liver for those methods (Stoner, 1957).

With this evidence against the ischaemic theory of the production of centrilobular necrosis, it is interesting to find that the compounds used (CCl_4, dimethylnitrosamine and sodium fluoroacetate) depressed the liver temperature.

Although this suggests interference with the oxidative metabolism of the liver there was no simple relationship between this depression and the extent of the necrosis. Sodium fluoroacetate produced the greatest fall in liver temperature, but little necrosis, and a similar severe fall in liver temperature was produced by intramuscular injections of ether without necrosis. Although the centrilobular necrosis produced in the rat by the compounds just mentioned is more probably due to direct interference with cellular metabolism than to hepatic ischaemia, this need not be the explanation in every case or every species. At the moment each toxin must be considered separately. While it is usually possible to apply the results of animal experiments to man when they give evidence of such broad changes as necrosis, for anatomical reasons (Elias & Popper, 1955) it may be very difficult to apply the detailed results directly to human pathology.

Senecio poisoning may be an example of this. As seen in South Africa by Selzer & Parker (1951), it was a rapidly developing condition with early signs of portal obstruction. Histologically, in addition to haemorrhagic centrilobular liver necrosis, a characteristic feature was occlusion of the small hepatic veins. Bras & Hill (1956) have suggested that the " veno-occlusive disease of the liver " seen in the Caribbean area may also be due to senecio poisoning and that the centrilobular necrosis is secondary to the blockage of the small veins in the acute stage. The pyrrolizidine (senecio) alkaloids also cause hepatic necrosis with changes in the small hepatic veins in the rat (retrorsine—Davidson (1935); Selzer, Parker & Sapeika (1951); isatidine—Schoental, Head & Peacock (1954); lasiocarpine—Schoental & Magee (1957)), but the vascular changes are rather different and their importance is difficult to assess. Davidson (1935) and Harris, Anderson & Chen (1942) thought that the necrosis was secondary to the vascular damage, but Selzer *et al.* (1951) found changes in the liver cells before those in the vessels; and lasiocarpine depressed the liver temperature within 30 min. of the intramuscular injection of a fatal dose, many hours before the hepatic blood flow was altered (Stoner, unpublished). At least in the rat, therefore, there would appear to be evidence for a direct action of these compounds on the liver cells.

Although centrilobular necrosis is the commonest toxic liver injury, recent work on other types of zonal necrosis should not be overlooked.

The intravenous injection of beryllium salts produces

hepatic necrosis which initially has a mid-zonal distribution (Scott, 1948; Aldridge, Barnes & Denz, 1949), and the mechanism of this has now been clarified by Cheng (1956) using improved histochemical and transillumination methods (Jarrett, 1954). In plasma the beryllium probably exists as colloidal compounds, and as these particles circulate through the liver they are removed by the Kupffer cells, especially those of the afferent terminal vessels where most of the particulate matter reaching the liver is removed. The accumulation of beryllium compounds in the Kupffer cells is followed by destruction of the latter. The beryllium compounds are then free to diffuse to the neighbouring parenchymal cells which in turn become necrotic. It is this necrosis around the terminal afferent vessels which gives the " mid-zonal " appearance of the lesion. While the biochemical events leading to these structural changes are not known, Cheng's work shows that they form the primary lesion to which the circulatory changes in this type of necrosis are secondary. This was borne out in experiments with the heated thermocouple where the step-wise production of beryllium necrosis was clearly seen (Stoner, 1956).

The periportal necrosis produced by allyl formate is chiefly of theoretical interest. Eppinger (1937) thought its action was confined to blood vessels and that the hepatic necrosis was secondary to this. Himsworth (1947) advanced the view that it had a direct toxic effect on liver cells but that the central cells were protected by the sinusoidal dilatation which is a feature of this poison. Current German work (Fleckenstein, 1944; Kessel, Körtge & Pezold, 1954) supports the view that allyl formate is a metabolic poison, as does the finding that the fall in liver temperature it produces is directly proportional to the log. dose (Stoner, 1956). Although its main action appears to be on cellular metabolism, it has an effect on liver blood flow, the importance of which is difficult to assess. After necrotizing doses the hepatic blood flow is reduced; this makes it doubtful if dilatation of the sinusoids protects the central cells and raises the possibility of a fall in blood flow's being concerned in the production of the lesion.

From this account it will be seen that the current tendency is to attribute all types of toxic liver injury to direct interference with the metabolism of the liver cells by the toxic agent.

4. Specificity of Action and the Metabolism of Toxic Agents

A striking feature of some of the poisons under discussion is the way in which they damage the liver while having little effect on other organs. Examples of such compounds are butter yellow (*p*-dimethylaminoazobenzene) (Orr & Price, 1948); thioacetamide (Ambrose, DeEds & Rather, 1949); and dimethylnitrosamine (Barnes & Magee, 1954). Where damage is produced outside the liver, it may be due to a direct action of the toxic agent, as in the case of ethionine (Popper, de la Huerga & Koch-Weser, 1954), or it may be secondary to the liver injury. It is important to attempt to distinguish between these two possibilities although both factors may play a part, as in the renal lesions produced by CCl_4 (Oliver, MacDowell & Tracy, 1951).

The metabolism of several of the hepatotoxic agents has been studied, and consideration of their fate in the body suggests reasons for the specificity of action of some of them. Miller & Miller (1953) have shown that the carcinogenic azo dyes undergo extensive chemical alteration in vivo, and Mueller & Miller (1948) further showed that breakdown of these compounds can occur in vitro in the presence of slices and cell-free preparations of liver but not of other tissues. Dimethylnitrosamine is rapidly metabolized by the intact rat (Dutton & Heath, 1956) but not by the totally hepatectomized rat (Magee, 1956); and Vandekar & Magee (unpublished) have found that metabolism also occurs in vitro, again only in the presence of liver tissue. As for the azo dyes (Kensler, Miller & Hyatt, 1956; Mueller & Miller, 1953), activity in vitro was confined to the microsome plus supernatant fraction of the homogenate, with a requirement for di- and triphosphopyridine nucleotides. Carbon tetrachloride was shown by McCollister, Beamer, Atchison & Spencer (1951) to be metabolized in monkeys, and Nygaard, Eldjarn & Nakken (1954) found evidence of rapid breakdown of thioacetamide after administration of this compound labelled with ^{35}S to rats. In view of the special capacity of the liver to metabolize foreign compounds, which has recently been clarified by Brodie and his school (Brodie, Axelrod, Cooper, Gaudette, La Du, Mitoma & Udenfriend, 1955), many of the hepatotoxic compounds may be broken down mainly or perhaps only by the liver. If this is the case, a possible explanation for their specific action is that a metabolite, rather than the compound itself, is the real toxic agent. Such a view has been advanced by Davidson (1935) to explain the action of the pyrrolizidine (senecio) alkaloid retrorsine, by Miller & Miller (1953) for azo-dye carcinogenesis, by Nygaard *et al.* (1954) for thioacetamide, and by Magee (1956) for dimethylnitrosamine. The last compound was shown to be rapidly distributed throughout the total body-water after injection, therefore it must penetrate into the cells of the other tissues as well as the liver. Since many fundamental biochemical processes are common to most tissues, and enzyme inhibitors such as cyanide in general exert their effects on enzyme systems regardless of the tissue, it seems that the theory of a locally produced toxic metabolite is worthy of serious consideration. It is now well established that metabolic alteration of foreign compounds is not necessarily beneficial, and the whole idea of detoxification has come under review since the concept of the " lethal synthesis " (Peters, 1952). However, the mechanism of the predominant action of certain poisons on the liver is still unsolved, since they may act on physico-chemical systems peculiar to the liver or on systems common to other tissues but whose proper function is specially important for the integrity of the liver cell (cf. Hicks, 1950a, 1950b). Apart from the question of specificity, the study of the metabolism of hepatotoxic compounds is of paramount importance because, if a toxic metabolite is involved, its chemical nature must be known before the biochemical lesion can be elucidated.

5. The Biochemical Mechanism of Action of Liver Poisons

A large amount of biochemical work has been done in which changes in the liver have been observed after the administration of liver poisons. Much of this work, however, is of rather limited value because of failure to take into account certain anatomical and pathological considerations. In the first place, as pointed out by Himsworth (1947), the liver is a composite organ, with several different cell types, so that all biochemical studies in which the liver tissue is studied en masse can only give an over-all picture. The situation is further complicated by the characteristically zonal distribution of the cellular damage, and the extraordinary capacity of the liver for regeneration. In the case of CCl_4 poisoning

this regenerative activity is seen in the periportal parenchymal cells, while the cells surrounding the central veins are necrotic and in process of being cleared away by phagocytes. Biochemical measurements on such livers, therefore, will be the resultant of cell death and cell regeneration and are very difficult to interpret. It is therefore essential for biochemical changes to be correlated with the histological appearance of the liver, and histochemical methods are of great value in making such correlations. These remarks apply of course to all studies in chemical pathology. Good examples of the combined morphological and chemical attack are the studies of Stowell and his colleagues (Stowell, 1949; Tsuboi, Stowell & Lee, 1951), of Campbell & Kosterlitz (1952) and of Hoffman, Himes, Lapan, Riszki & Post (1955). The significance of a biochemical change in a poisoned liver is very difficult to assess, even with careful histological correlation, since it may represent a specific interference in cell function due to the toxin, or only indicate a phase in the death of the cell. Thus many unspecific biochemical changes may be found which are merely secondary to the true " biochemical lesion " as defined by Peters (1936). Limitation of space makes any attempt at a complete survey of the literature impossible, and the work for discussion has been selected where early changes after a single dose of the toxin have been described and where theories of the mechanism of action have been put forward.

Biochemical changes in the liver after CCl_4 have been studied very extensively, sometimes with conflicting results. The oxygen uptake of liver slices in vitro has been reported as raised by some workers (Ennor, 1942; Richter, 1951) and lowered by others (Christie & Judah, 1954; Niwa, 1954). These differences may well be related to the morphological state of the liver at the time of the experiment. Christie and Judah investigated the behaviour of respiratory enzymes of liver from rats poisoned with CCl_4, using cell-free preparations in vitro, and found various defects in the oxidation of substrates of the tricarboxylic acid cycle. They concluded that the mechanism of action of the compound is a direct physical attack on the liver mitochondria, with consequent increase in permeability and loss of soluble co-factors, notably diphosphopyridine nucleotide. These authors also separated a fraction of fatty globules from homogenates of the poisoned livers, and showed that these globules were capable of oxidizing succinate, thus providing evidence in favour of the old theory that some of the globules seen in fatty livers are, in fact, mitochondria which have become swollen and ensphered by the accumulation of fat within them. The rounding of liver mitochondria as an early sign of liver damage has been known for many years (Duthie, cited by Cameron & Karunaratne, 1936) and has recently been studied in the electron microscope by Dianzani & Bahr (1954) using isolated mitochondria. Dianzani (1954) described uncoupling of oxidative phosphorylation in the livers of rats treated with CCl_4 and with phosphorus. However, nearly all his animals received repeated doses, probably large enough to cause necrosis, so his findings are of doubtful relevance to the biochemical lesion. In later work, Dianzani (1955) confirmed the conclusion of Christie & Judah (1954) on the loss of pyridine nucleotides from the mitochondria of CCl_4-poisoned livers by direct analysis. A loss of coenzyme A from CCl_4-poisoned livers has been reported by Heim, Leuschner & Ott (1956). The suggestion has been made (cf. Drill, 1952) that the chlorinated hydrocarbons damage the liver by reaction with sulphydryl groups. However, Eden & Harrison (1955) found no evidence of combination of CCl_4 with glutathione in vitro, and in vivo they found a rise in the concentration of glutathione in rat liver 24 hr. after CCl_4; this they attributed to hepatic regeneration.

Gallagher, Gupta, Judah & Rees (1956) have studied biochemical changes in rat liver in thioacetamide poisoning. They suggest that the primary lesion is a defect in permeability of the hepatic cell wall, permitting the entry of calcium which then inhibits oxidative phosphorylation. Thioacetamide had no effect on the respiration of liver mitochondria when it was added in vitro. This would be consistent with the view that a metabolite is the true toxic substance. From their studies on autolysis of liver, the same authors (Gallagher, Judah & Rees, 1956) concluded that ". . . an understanding of the action of agents causing necrosis must be based on determining the manner in which they inhibit the synthesis of ATP in the cell ". Interference with phosphorylation mechanisms has been suggested by Fonnesu & Severi (1956) to explain cloudy swelling.

Evidence is now accumulating that some of the hepatotoxins may act by interference with protein metabolism. Popper *et al.* (1954) have put forward the idea of a conditioned amino-acid deficiency in bromobenzene poisoning and in the liver lesion due to the abnormal amino acid ethionine. Bromobenzene causes acute centrilobular necrosis which can be largely prevented by the administration of methionine. Part of the injected dose of bromobenzene is excreted in the urine as mercapturic acid, and Popper *et al.* (1954) suggested that this process depletes the free amino-acid pool of the liver of sulphur-containing amino acids, causing death of the liver cells. A similar mechanism was suggested to explain the lesions due to ethionine. There is also evidence for the incorporation of ethionine into tissue proteins, with the formation of an abnormal protein (Levine & Tarver, 1951; Gross & Tarver, 1955).

The work of Miller & Miller (1947) on the formation of a dye-protein compound in the livers of rats fed carcinogenic azo dyes is now well known. This phenomenon is found only in the liver (and in plasma proteins), and the dye is probably bound to the protein by covalent bonds. The formation of similar firmly bound complexes of 2-acetamidofluorene and liver protein was suggested by Weisberger, Weisberger & Morris (1953), and recent work by Gutmann, Peters & Burtle (1956) has indicated that the compound combined with the protein is not 2-acetamidofluorene itself but a metabolite, the hydroxy derivative. The acute necrotic lesion due to azo dyes (Orr & Price, 1948) has received far less attention than the various stages of liver carcinogenesis, but very recently Hultin (1956a) has demonstrated the formation of the dye-protein compound as early as 3 hr. after administration. During the early period of rapid uptake, the proteins of the microsome fractions had a much higher content of bound dye than those of the other fractions, and Hultin suggested a possible interference with protein metabolism in view of the role of the microsomes in protein biosynthesis (see Borsook, 1956). In later work, however, he suggested that the azo dye incorporation is mediated by a mechanism independent of that for the incorporation of amino acids (Hultin, 1956b). In recent work Magee (1957) has shown that the incorporation of isotopically labelled amino acids into rat liver in vivo is markedly reduced 3 hr. after an oral dose of dimethylnitrosamine, but that the specific activity of the free amino acids of the liver is unchanged. Incorporation into kidney,

spleen and pancreas was not affected. At this time histological evidence of damage to the liver is minimal or absent. Wahi, Tandon & Bharadwaj (1955) found an early rise in the free amino-acid concentration in CCl_4-poisoned livers, and postulated that this might be due to rapid proteolysis as well as to blockage in amino-acid metabolism.

It appears, therefore, that there are two general schools of thought on the mechanism of toxic liver injury. One suggests an interference with energy production in the cell as the fundamental derangement, while the other suggests a primary disorder of protein metabolism. These views are not necessarily incompatible, since recent work on protein biosynthesis (Borsook, 1956) has shown that both free amino acids and an energy source are required for peptide bond formation. However, no convincing biochemical lesion has yet been demonstrated in any type of toxic liver injury. As stated above, many of the hepatotoxins have also been shown to be carcinogens, and it is possible that the primary reaction between the toxin itself or a toxic metabolite and a cell constituent may be the same, whether the compound is given in a large dose causing necrosis or in repeated smaller doses ultimately producing tumours. If this is true, study of the very early stages of the acute lesion may be valuable in the elucidation of the chronic one.

6. Conclusions

It is clear that, although we are still a long way from understanding toxic liver injury, its problems have stimulated a large amount of experimental work on the pathology of the liver. This has, in turn, demanded a fuller knowledge of its anatomy, physiology and biochemistry; and some of the toxic compounds have formed the tools for the acquisition of this new knowledge. Current thinking supports the view that toxic liver injury results from a direct interference with the biochemical machinery of the liver cell by the toxic agent. A complete explanation of the injury produced by any of these agents would greatly increase our knowledge of the normal function of the liver.

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NUTRITIONAL LIVER DAMAGE IN MAN

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1 Atrophy
2 Fatty liver
3 Cirrhosis
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References

The belief that liver damage in man can be caused by a deficient diet is not new, but until recent years it has been supported by very little positive evidence. Interest in the problem has been stimulated, and knowledge very greatly increased, by three developments that seem in their origin to have been quite unconnected. The first is the realization that liver disease is unduly common in many under-developed and tropical countries. This point was very clearly brought out at the International Congress on Geographical Pathology in 1931. The second factor is the development of the technique of liver biopsy, mainly for diagnosis in virus hepatitis, by Iversen & Roholm (1939) in Copenhagen, by Sherlock (1945) in London, and by Volwiler (1947) and others in the USA. Most important of all, however, is the stimulus derived from the experimental production of dietary liver injury in animals.

This experimental work is referred to elsewhere in this Bulletin, and has been summarized in a number of recent publications, e.g. Himsworth (1950); Miner (1954). From the point of view of human pathology the most important result of the animal work, most of which has been done on the rat, is the definition of two distinct types of dietary liver injury: fatty infiltration, produced, as the Toronto group showed, by deficiency of choline and its precursors (Best & Huntsman, 1932),[1] and necrosis of the liver, caused by diets low in sulphur-containing amino acids (György & Goldblatt, 1939, 1941; Daft, Sebrell & Lillie, 1942; Himsworth & Glynn, 1944a, 1944b). An apparently identical necrosis has also been produced by deficiency of vitamin E (Schwarz, 1944; György, 1947). Both types of lesion can, under appropriate circumstances, lead to fibrosis of the liver. Fatty infiltration, if sufficiently severe and long continued, is followed by a diffuse proliferation of fibrous tissue, whereas necrosis, if not fatal, leads to coarse scarring and nodule formation.

Both these pathological sequences can be prevented by diets rich in protein. The amino acid methionine plays a dual role: it protects against fatty change by acting as a donor of methyl groups for the synthesis of choline, and it protects against necrosis in virtue of its sulphur content. It is therefore a fact of some significance that in human dietaries methionine is one of the amino acids most likely to be limiting or deficient.

Before these dietary experiments the only way of producing experimental liver injury was by exposure of the animal to hepatotoxic agents.[2] Consequently necrosis and cirrhosis had been looked upon as acute and chronic forms of hepatic injury, often in response to the same toxic substance, and differing in degree rather than in kind. The nutritional experiments, however, provided a rational basis for the separation, long advocated by some pathologists, notably Mallory (1911), of two basically different forms of human liver disease: (i) a process which, without massive necrosis, gradually progresses towards diffuse cirrhosis of the Laennec type, and (ii) massive necrosis, giving rise to the morbid anatomical picture of post-necrotic scarring.

The problem may therefore be posed very simply: what is the evidence that either of these sequences can be produced in man by dietary means? In trying to answer this question, we shall consider one by one the various types of liver lesion that have been found in malnourished communities, particularly in tropical countries. It must, however, be borne in

[1] See also Best, Lucas & Ridout, *Brit. med. Bull.* 1956, **12**, 9.—ED.
[2] See Stoner & Magee, p. 102 of this number of the Bulletin.—ED.

EXPERIMENTAL STUDIES ON TOXIC LIVER INJURY

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mind that malnutrition may occur as a conditioned or secondary state. In alcoholism, for instance, there is often a deficient intake of all nutrients except calories. Therefore, as Patek & Post (1941) have suggested, "alcoholic" cirrhosis might be regarded as a conditioned deficiency disease, like alcoholic beriberi.

1. Atrophy

In starvation and hunger oedema, such as occurred in Europe during and after the last war, and in marasmic infants, the liver tends to be small, but shows little in the way of specific histological changes (Ühlinger, 1948). In animals, as Davidson describes elsewhere in this Bulletin, p. 77, starvation or a low-protein diet causes a severe loss of several cytoplasmic constituents of the liver cell—protein, phospholipid, and ribonucleic acid. The loss of liver protein may amount to 40% in a few days. This effect, which from the pathological point of view may be regarded as an atrophy, is difficult to demonstrate histologically. Thus Sherlock & Walshe (1951), in biopsy studies in cases of famine oedema in Germany, found a normal histological picture, except for accumulation of pigments, and normal liver function tests. In Jamaica we have shown that the liver weight is reduced and that chemically there is a large reduction of liver protein in malnourished human infants, quite comparable to that produced experimentally (Waterlow & Weisz, 1956; Bras, Waterlow & DePass, 1956). In livers that were not fatty, the histological picture showed nothing striking, except perhaps a reduction in the size of the liver cells. This loss of protein undoubtedly involves a loss of liver enzymes, and though for a long time it may be reversible, it can hardly be without physiological significance. Experimentally it has been shown that the atrophic or malnourished liver is more vulnerable to injury by poisons (Miller & Whipple, 1940; Allison, 1957; György, Goldblatt & Bras, unpublished observations, 1956). Therefore although this type of atrophy is an inconspicuous lesion from the histological point of view, it may perhaps be an important conditioning factor, even when present in minor degrees. This is a point to which we shall return later.

2. Fatty Liver

Perhaps the commonest clinical manifestation of malnutrition throughout the world at the present time is the syndrome called kwashiorkor, which develops in infants fed a starchy diet deficient in protein (Trowell, Davies & Dean, 1954). In fatal cases of this disease the liver is typically very fatty. When this was first noticed, it was thought to be a non-specific change of no significance. Gillman & Gillman (1945a), in a pioneer series of biopsy studies in Johannesburg, showed that the fatty liver occurred in vivo, and was not just a terminal event; it was not related to infection, and it disappeared with dietary treatment. Waterlow (1948), impressed by the fact that in the West Indies fatty liver often occurred without the other features of kwashiorkor, described a group of cases under the title "fatty liver disease". Since then studies in almost all the countries of the world where kwashiorkor occurs have confirmed that fatty metamorphosis of the liver, of greater or less degree, is a characteristic feature of the syndrome.

A good case can therefore be made that this is an example of dietary liver injury in man. There is no doubt that kwashiorkor is a nutritional disease; the fatty liver, like the other features, responds to treatment by diet alone. The fact that the main cause of kwashiorkor is deficiency of dietary protein provides an encouraging analogy with fatty liver in the rat. Nevertheless, rigorous proof, such as that laid down by Koch's postulates in bacterial disease, is difficult to achieve. The fatty liver of kwashiorkor is an infiltration in which each cell—or the majority of liver cells—contains a single large fat droplet. In amount, fat represents 30–50% of the wet weight of the organ (Waterlow & Weisz, 1956; Waterlow, Bras & DePass, 1957). This lesion is quite distinct morphologically from the type of fatty change with multiple small droplets that is frequently seen in acute conditions, and that seems to be a non-specific response to toxins or anoxia. But even the large-droplet form of infiltration is certainly not specific to kwashiorkor. As is very well known, it occurs in alcoholism, in some cases of diabetes (Connor, 1938), and sometimes in infections, notably gastro-enteritis and tuberculosis (Girard, Plauchu & Loisy, 1953). Secondly, fatty liver cannot be regarded as necessarily the central or most important feature of the kwashiorkor syndrome. In fact, recent work in Jamaica suggests that, contrary to our earlier view, it is a kind of epiphenomenon: the degree of infiltration seems to bear very little relation to the clinical severity of the disease (Waterlow, 1955; Bras *et al.* 1956), and it causes very little disturbance in tests of liver function.

The exact status of fatty liver as a form of nutritional liver injury therefore cannot be settled until its cause is known in more detail. The therapeutic trials made by Gillman & Gillman (1945b) in South Africa, and by us in the West Indies (Waterlow, 1948) were necessarily crude, but they showed no clear-cut response to the lipotropic factors, such as choline and methionine, that are so active in the rat. By universal agreement the most effective treatment for kwashiorkor is milk; when an adequate intake is secured, the fatty liver rapidly becomes normal again. Preliminary reports suggest that both purified casein and an amino-acid mixture are also effective (Brock, Hansen, Howe, Pretorius, Davel & Hendrickse, 1955).

The response to protein but not to choline shows that the cause of the fatty liver in kwashiorkor is not simply deficiency of known lipotropic factors. There is, moreover, an anatomical difference between the two conditions: in kwashiorkor the accumulation of fat occurs first in the peripheral part of the liver lobule (Davies, 1948a), whereas in the choline-deficient rat it is centrilobular. Recently, however, several groups of workers, in Canada, in India, and in the USA, have succeeded in producing peripheral fatty infiltration in the livers of rats on low-protein diets, even in the presence of adequate choline (Best, Hartroft, Lucas & Ridout, 1955; Ramalingaswami, Sriramachari & Patwardhan, cited by Gopalan & Ramalingaswami, 1955; Harper, Benton, Winje & Elvehjem, 1954; Shils, De Giovanni & Stewart, 1955). This experimental work therefore fits in well with the human findings: it shows that deficiency of protein per se can promote excess deposition of fat in the liver, but the mechanism by which this occurs is not known.

Another aetiological approach has been suggested by Davies in Uganda. In kwashiorkor there is an atrophy of the external secretory cells of the pancreas (Davies, 1948a; Waterlow, 1948). Davies suggested that this might be the primary lesion, causing a fatty liver like that which many years ago Macleod and his colleagues found in depancreatized dogs (Allan, Bowie, Macleod & Robinson, 1924). (It may be

recalled that it was this observation that led to the discovery by Best of the lipotropic action of choline.) We have recently demonstrated a statistical correlation between degranulation and atrophy of the pancreatic acinar cells, and the degree of fatty infiltration of the liver (Bras, 1955a; Bras *et al.* 1956).

The fatty liver of kwashiorkor is the liver lesion for which a dietary origin can be claimed with most confidence; even so, we must accept the fact that it is not a specific change, and its mode of production is still unknown.

3. Cirrhosis

Cirrhosis is defined pathologically as a lesion involving distortion of the architecture of the liver, fibrous tissue formation, and regeneration of liver cells. It is well recognized that with few exceptions cirrhosis is more common in many parts of the tropics and subtropics than in Europe or North America (Josselin de Jong, 1931). In the incidence figures, published from tropical countries, usually no distinction has been made between the various types of cirrhosis. In part this reflects the difficulty of classifying liver damage once it has progressed to any degree of severity, since a variety of morphogenetic conditions can produce a similar end-result.

The morphological types of cirrhosis whose possible relation to malnutrition must be considered are as follows.

a. Laennec's Cirrhosis (Portal Cirrhosis, Diffuse Finely Granular Cirrhosis, Diffuse Septal Cirrhosis)

This is probably the commonest form of cirrhosis in the tropics. There can be no question of any relation to alcoholism, and from the geographical distribution parasitic infestations, such as schistosomiasis, can also be excluded. Could this cirrhosis be a nutritional lesion, developing as a sequel to fatty infiltration, as in the rat and the dog (Chaikoff & Connor, 1940)?

Geographically there is a fairly close correspondence between the incidence of kwashiorkor and of Laennec's cirrhosis (Brock, 1954). Nevertheless, there are discrepancies which cannot be ignored or put down to incomplete data. In Curacao, for instance, fatty liver was until recently common, but there was no undue incidence of cirrhosis (van der Sar, 1951). The same seems to hold for Brazil, at least in the region of Rio de Janeiro (Carvalho, Pinto, Schmidt, Potsch & Costa, 1945; Fraga, personal communication, 1953). Conversely, in the Gambia, West Africa, cirrhosis was reported to be common even in children, but fatty livers were seldom found (Walters & Waterlow, 1954; Charmot, 1954). Even when there is a geographical correlation, it is unsafe to assume a causal connexion—a point that has been brought out very clearly by our experience in Jamaica (see below).

Another line of approach is to demonstrate histologically a progression from fat to fibrosis by serial studies, as has been done in the rat (Glynn, Himsworth & Lindan, 1948; Hartroft, 1949; Lillie, Ashburn, Sebrell, Daft & Lowry, 1942). No one has yet succeeded in showing this in human beings. In follow-up studies of kwashiorkor we have never yet found evidence of a fatty liver becoming cirrhotic (Bras, 1955b), and this is also the experience of Brock in Cape Town (Brock, personal communication, 1956). So far, however, cases have not been followed for very many years.

Evidence can also be obtained by cross-sectional studies of different age-groups from infancy onwards. This method, however, has its pitfalls. When fatty liver and cirrhosis were both found to be common in Jamaica, the conclusion was drawn that the one arose from the other (Waterlow, 1948). It is now clear that this was a fallacy: childhood cirrhosis in Jamaica is the result of a totally different lesion—veno-occlusive disease (Bras, Jelliffe & Stuart, 1954; Rhodes, 1955a). Davies (1950) in Uganda believed that he could demonstrate every stage in the progression from fatty liver to cirrhosis. More recently, however, he has modified this view (Davies, personal communication, 1956). Gillman & Gillman (1951) have argued strongly against the existence of a causal relation between the two lesions. This was also the conclusion reached by Dible (1951) in his studies of cirrhosis in the United Kingdom, and by Cachera and his colleagues in Paris in their work on alcoholic fatty liver (Cachera, Lamotte & Darnis, 1953).

If cirrhosis is in some way a sequel to fatty liver, a puzzling feature that has to be explained is the latent period. Although in the tropics cirrhosis occurs earlier than in temperate countries, it is still typically a disease of adult life. What is happening during the long interval after recovery from kwashiorkor in infancy? Information about the condition of the liver during later childhood and adolescence is rather scanty. Davies (1948a) showed that in cases of kwashiorkor, when the fat has disappeared from the liver, there remains a mild periportal or "stellate" fibrosis, which tends to be more marked the older the patient. In Africa this lesion is very commonly found at autopsy, whatever the cause of death. It was described in some detail by Vint (1931) in Kenya; he called it a monolobular cirrhosis, and thought that it might be caused by toxins produced by intestinal parasites. According to present conceptions, this lesion would not be classified as a cirrhosis, since the fibrosis is localized, and does not distort the normal architecture. Perhaps it may predispose to cirrhosis, but it is certainly not an invariable precursor. It is noteworthy that in three countries where a true cirrhosis has been found to occur rather commonly in children—Jamaica, India, and the Gambia—in no case does the process begin with fatty infiltration (Bras & Hill, 1956; Indian Council of Medical Research, 1955; Walters & Waterlow, 1954). On the whole, therefore, it seems that the case for a "post-fatty-liver cirrhosis", which ten years ago was such an attractive hypothesis, cannot now be upheld.

b. Post-Necrotic Scarring (Post-Collapse Cirrhosis, Toxic Cirrhosis, Coarse Nodular Cirrhosis or Multiple Nodular Hyperplasia, Multiple Nodular Cirrhosis)

This form of cirrhosis is a sequel to massive necrosis of the liver. Histologically it is similar to the massive necrosis produced in the rat by deficiency of sulphur-containing amino acids or of vitamin E (Himsworth & Glynn, 1944a; György, 1947). However, there is no evidence that in man nutritional deficiency is ever a direct cause of massive hepatic necrosis. The conditions for the production of this lesion experimentally are somewhat specialized, and it may be that man never eats, nor could survive on, a diet unbalanced in this particular way. Himsworth & Glynn (1944b) suggested that deficiency of sulphur-containing amino acids might be a conditioning factor in some cases of acute or subacute necrosis of the liver, as in pregnancy or poisoning. That treatment with methionine proved ineffective does not rule out this theory, because necrosis is already established by the time treatment is begun.

In temperate zones post-necrotic scarring is most often the result of preceding virus infection, but in the tropics the position may well be more complicated. It is possible that in the malnourished individual the reactions of the liver are totally different, either in degree or in kind, from those that obtain in better-fed people. Several investigators have observed that the damage caused by infective hepatitis is more severe in the tropics than in Europe or North America. Findlay (1948) found that the mortality was 15 times higher in West African than in European troops. Fernando and his colleagues reported that in Ceylon it was 100 times higher than in temperate countries (Fernando & Thanabalasunderam, 1951; Fernando, Mendoza & Rajasuriya, 1948). Pathological studies have shown that the necrosis is more extensive, and cirrhosis a commoner sequel (Straub & Schaberg, 1950; Bergeret & Roulet, 1947). It is probable that this contributes to the over-all increase in the incidence of cirrhosis in tropical countries.

These observations suggest that malnutrition may act on the liver indirectly, making it more vulnerable to toxic and infective agents. This idea will be considered in more detail below.

c. Veno-Occlusive Disease (VOD)

Several workers in Jamaica have drawn attention to the prevalence there of ascites and cirrhosis in quite young children (McFarlane & Branday, 1945; Royes, 1948). The essential pathological features have been summarized by Bras & Hill (1956). Occlusion of branches of the hepatic veins apparently leads to centrilobular parenchymatous damage, which is followed by fibrosis and eventually by a non-portal cirrhosis.

The disease is seen occasionally in temperate countries; the symptoms resemble those found in thrombosis of the hepatic veins (Chiari's syndrome). Sporadic cases have been reported by European authors as endophlebitis obliterans (Rössle, 1933; Teilum, 1949). Selzer & Parker (1951) reported similar changes in patients who were apparently exposed to the toxic action of Senecio. We have been able to convince ourselves that the disease occurring in cattle and horses from poisoning by species of Senecio and Crotalaria—as well as perhaps by other weeds—is histologically indistinguishable from VOD as seen in Jamaican children and adults (Bras & Berry, 1956). It is a very real possibility that this non-portal cirrhosis which is so commonly seen in Jamaica may be caused by the action of plant toxins, since the people are in the habit of taking watery extracts from a variety of plants, including Senecio and Crotalaria, for medicinal and other purposes. Animal experiments (to be reported in detail later) indicate that there is some protective action of protein against the toxic effects of these plant infusions. Furthermore, dietary surveys by Rhodes (1955b) have shown that patients with VOD were existing on a protein-deficient diet.

d. Childhood Cirrhosis in India

This disease typically occurs in children between one and three years old. Unfortunately, morbid anatomical studies are rather scanty. Radhakrishna Rao (1935) concluded that it was a toxic cirrhosis and drew attention to occlusion of hepatic veins. More recent studies in India have failed to confirm this finding, and have suggested that the initial lesion is a diffuse or focal necrosis (Indian Council of Medical Research, 1955). However, we have had the opportunity to examine tissues from India, and in a number of them there was unquestionably a picture indistinguishable from that of chronic venous occlusive disease in Jamaica (Jelliffe & Bras, in preparation). The characteristic picture of acute VOD so frequently seen in Jamaica was not observed, either clinically or histologically.

The cause has been suspected by the Indian workers to be either a toxin or a virus, but neither has yet been demonstrated. It seems unlikely that this is a dietary disease, because it occurs in well-to-do Brahmins, and is seldom found among the poor classes, who suffer from kwashiorkor. In the words of the Indian Sub-Committee on Liver Diseases (Indian Council of Medical Research, 1955): "If [malnutrition] does play a part, it alone is not a sufficient cause, but may act as an important contributory factor under certain conditions."

e. Cirrhosis Associated with Cytosiderosis in South Africa

Gillman & Gillman (1951) gave the name cytosiderosis to a deposition of iron-containing pigment in the liver, usually but not always accompanied by fibrosis or frank cirrhosis. There has been some confusion over terminology (Brock, 1955), but the condition is probably not the same as idiopathic haemochromatosis (Higginson, Gerritsen & Walker, 1953). It is thought that the excess iron comes from the iron cooking-pots used by the South African Bantu. The Gillmans suggested that there is a breakdown of the normal intestinal barrier to the absorption of iron. They do not believe that siderosis, any more than fatty infiltration, is a cause of fibrosis or cirrhosis, but regard the three lesions as results of distinct and separate metabolic disturbances.

f. "Malarial" Cirrhosis

Malaria has long been regarded as a possible cause of hepatic cirrhosis. In recent years the theory has fallen into disrepute, particularly among British workers. A variant of it has been revived as a result of observations made in the Gambia (Walters & Waterlow, 1954). Cirrhosis and fibrosis of the liver were found to be rather common in children, although malnutrition was less severe than in many parts of the world, and fatty livers were rare. On the other hand, malaria is highly endemic in the Gambia, and all children are affected from the first year of life. Experience in other malarious countries, such as Greece, suggests that malaria alone seldom if ever causes a true cirrhosis, but it seemed possible that a combination of malaria and malnutrition might have a much more severe effect than either alone. It was suggested that perhaps cirrhosis may result when the two main tissues of the liver are simultaneously attacked, the mesenchymatous elements being stimulated by malarial infection, and the parenchyma functionally altered by malnutrition.

Such a dual theory of the aetiology of cirrhosis has certain advantages: the need for two factors to coincide helps to explain many discrepant facts in incidence and aetiology. According to circumstances, toxic, infective or parasitic agents, when combined with malnutrition, may play a causative role. We have put forward possible examples of each of these. The weakness of the theory is that it postulates a nutritional lesion, presumably of the parenchyma, which has not been visualized microscopically, and which therefore remains hypothetical until established by functional or

cytological studies. In the Gambian cases the only histological change found in the liver cells, apart from a few fat droplets, was a " piece-meal " degeneration of seattered cells, too slight to be regarded by itself as a significant precursor of cirrhosis. A similar piece-meal degeneration has been described in the livers of infants recovering from kwashiorkor (Bras, 1955c).

4. Carcinoma of the Liver

In Indonesia (Snijders & Straub, 1923; Bonne, 1935), in Africa (Berman, 1940; Davies, 1948b), and perhaps in other tropical regions, there is an inordinately high incidence of primary cancer of the liver. For instance, in Uganda, Davies found that carcinoma of the liver formed 20% of all malignant neoplasms. In Johannesburg, Oettlé & Higginson (1956) record that among the Bantu the rate of primary carcinoma of the liver is 50–100 times higher than that expected from a Danish population of the same age and sex distribution. Since such rates are not found in the American negro (Steiner, 1954; Dorn, 1955), presumably this high relative[3] incidence in Africa must be caused by environmental factors. Among these malnutrition is an obvious possibility, acting either directly or indirectly.

Usually, although not invariably, carcinoma develops in a liver that is fibrotic or cirrhotic. It may be said, therefore, that cirrhosis predisposes to neoplasia; yet only a small proportion (3–17%) of cirrhotic livers become neoplastic. Therefore, even if malnutrition is accepted as a cause of cirrhosis, it does not follow that it also causes the extension to neoplasia. Admittedly the type of cirrhosis—whether diffuse or post-necrotic scarring—may have some influence, but the evidence on this is conflicting (Davies, 1952, 1955a; Higginson, 1955).

Experimentally, carcinoma of the liver and other organs has been produced in rats by diets deficient in choline (Salmon, Copeland & Burns, 1955), and changes suggestive of neoplasia follow the prolonged administration of ethionine, an antimetabolite of methionine (Popper & Bruce, 1955). Low-protein diets, however, are not effective, and calorie deficiency, as Tannenbaum has shown, tends to inhibit the development of tumours (Tannenbaum & Silverstone, 1953). Another possibility is that dietary conditions may predispose the liver to the action of carcinogenic agents. It is well known, for instance, that the composition of the diet has an important influence on the induction of hepatic tumours by the carcinogen butter yellow (Cramer, 1942; Opie, 1944); protein and riboflavin are protective. However, it must be admitted that up to the present time there is no evidence that naturally occurring carcinogenic substances are found in effective quantities in the diets of malnourished peoples.

There are some cytological observations that may have a bearing on the problem of liver cell cancer. According to Leuchtenberger, Leuchtenberger & Davis (1954), in the USA polyploid nuclei are not seen in the livers of children less than six years old. But in children recovering from kwashiorkor, large, apparently polyploid nuclei are often found in the liver, and dumb-bell shaped nuclei that are thought to be evidence of amitotic division (Bras, 1955c). It is not known for how long these changes persist, and no microspectrophotometric measurements have yet been made to confirm the visual impression of polyploidy. Observations of this kind, however, do suggest that nutritional influences can cause hyperplasia of the liver cell nucleus.

In this connexion an important suggestion has been made by Davies (1955b) and by Platt (1957): they have pointed out that in under-developed communities food supplies tend to be seasonal, with alternating cycles of shortage and plenty. It is possible that this kind of recurrent stimulus might have a much more damaging effect on the liver than a steady state of chronic deficiency.

5. Conclusion

The most securely established form of dietary liver injury in man is the fatty liver of kwashiorkor. This is the only case in which we have good evidence of a *direct* effect of diet on the liver. Striking though the picture is pathologically, this can hardly be called a disease, since recovery, both structural and functional, is the rule. On the other hand, the evidence that has accumulated in the last ten years does in our opinion support the view that deficient diets, such as are eaten by the peoples of under-developed countries, may have an *indirect* effect on the liver: they may predispose it to injury by other noxious agents—toxins, parasites or viruses, according to the *genius loci*. Such a theory is not new: it was put forward many years ago by Miller & Whipple (1940), and more recently with great force by Himsworth (1950); but it still has to be proved. A theory of dual aetiology of cirrhosis would reconcile many discrepancies, and bring some order into the bewildering diversity of pathological sequences and clinical syndromes of liver disease described in different parts of the world. But it is not enough to bring simplicity and order; a good theory must also point the way to observations by which it can be verified. The task of the future in this field is to discover and to define the functional and metabolic changes in the liver that precede structural changes, and that make it vulnerable to injury from every quarter. Until this has been done, the " conditioning " that has been postulated remains a pure hypothesis.

[3] Until recently no reliable data on true incidence, rate or prevalence have been available except for those of Findlay (1950). At a conference on cancer of the liver in Africa held in 1956 in Uganda, statistics were presented from several African territories, and will be published.

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PROTEIN METABOLISM AND THE LIVER

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A General considerations
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References

A. GENERAL CONSIDERATIONS

A. NEUBERGER

The necessary limitations of space make any attempt at a comprehensive review of the problem of interrelation between protein metabolism and liver disease impossible. Moreover the gaps in our existing knowledge make an orderly presentation difficult. This article presents limited aspects of the problem, in which further study is needed and which to the authors appear to be of special interest.

The liver receives about 70% of its blood supply through the portal vein and 30% through the hepatic artery, and the special position the liver occupies in the metabolism of the body stems to a large extent from the fact that it is interposed between the intestinal tract and the systemic circulation. More than 40 years ago Van Slyke & Meyer (1913a) showed that, during digestion of protein, the increased amino nitrogen in the portal blood was largely removed by the liver, and this was associated with an increased amino-acid content in that organ (Van Slyke, Cullen & McLean, 1915). Injection of amino acids led to a rapid and big increase of amino nitrogen in the liver, whilst the rise in muscle was delayed and not quite so great (Van Slyke & Meyer, 1913b). Experiments in which labelled glycine was used indicate that equilibration of free glycine of plasma with that of the liver is very fast, and it was suggested that this is due to the great permeability of the liver cell to glycine and to the high rate of blood flow (Henriques, Henriques & Neuberger, 1955). This fast rate of penetration is likely to apply to other amino acids, and it may thus be assumed that most of the digestion products of proteins will first be handled by the liver before being passed on to other tissues.

1. Amino-Acid Catabolism

The contribution of the liver to the breakdown of proteins and amino acids can be assessed either by an in-vitro study of the distribution of enzymes and enzyme complexes concerned with these catabolic reactions in various tissues or by examining the effects of impairment of liver function and of partial or complete removal of the liver on nitrogen metabolism. Breakdown of protein to amino acids can almost certainly take place in all cells, and enzymes catalysing deamination or transamination also have a very wide distribution. The activity of the oxaloacetic-glutamic transaminase is higher in heart muscle, skeletal muscle and in brain than it is in the liver of the rat (Cohen & Hekhuis, 1941). The important enzyme, L-glutamic dehydrogenase, occurs in high concentrations in liver and kidney, but its presence in brain, heart and skeletal muscle could also be demonstrated (Dewan, 1938). The formation and hydrolysis (Krebs, 1935) of glutamine also occur in tissues other than liver and thus there is no reason to suppose that deamination or transamination takes place exclusively or principally in the liver. On the other hand, the complete enzyme system responsible for synthesis of urea, discovered by Krebs & Henseleit (1932), has so far been found only in the liver, although the kidney contains enzymes catalysing one or two steps in this reaction, such as the conversion of citrulline to arginine (see Krebs, 1942; Ratner, 1954). Arginase, which promotes the last step in the synthesis of urea, is found in very high concentration in the liver, but is present in relatively small amounts in most other tissues; the first reaction, the conversion of ornithine to citrulline, is peculiar to the liver. It thus appears that the ultimate transformation of amino nitrogen into urea, the form in which the bulk of the nitrogen is excreted by the mammal, is an exclusive function of the liver.

Experimental work on the whole animal is in agreement with this conclusion. Most of the liver, possibly 90–95%, can be removed without any apparent impairment in the capacity of the body to deaminate amino acids or to produce urea. But after complete hepatectomy in dogs, blood amino nitrogen rises and urea output decreases sharply to almost zero (Bollman, Mann & Magath, 1924). Chromatographic analysis indicates that the increase in amino nitrogen is caused by a rise in concentration of all amino acids normally present in plasma (Flock, Mann & Bollman, 1951). Evisceration produces similar rises in the plasma concentration of amino acids (Frame & Russell, 1946; Ingle, Prestrud & Nezamis, 1947).

In most cases of disease of the liver in man, values for urea and amino nitrogen of blood are within normal limits and the changes which can be produced on feeding amino acids have been found to differ little from those observed in normal subjects (Witts, 1929; Kirk, 1936). This is in accordance with the results obtained with experimental animals, which indicate that the capacity of the liver to synthesize urea greatly exceeds demands normally made. It is only in terminal stages

of acute necrosis or other forms of liver failure or in fatal cases of poisoning with phosphorus or carbon tetrachloride that there is a marked rise in amino nitrogen and, occasionally, a fall in urea.

In addition to general reactions of amino acids such as deamination, transamination and urea synthesis, which concern all amino acids, there are specific catabolic pathways peculiar to certain amino acids, which may be exclusively associated with the liver; these may become deranged in disease at a stage when urea synthesis is not yet impaired. In many cases of liver disease there appears to be a relative excess of cystine and methionine both in plasma and urine (Dent & Walshe, 1951; Walshe, 1953). Although the in-vitro experiments of Pirie (1934) and of Medes (1939) indicate that the sulphur of cystine and cysteine can be oxidized to sulphate by several tissues of the rat, the in-vivo work of Folker, Chaikoff, Enteman & Tarver (1951a) shows that the oxidation of methionine sulphur occurs principally in the liver. The same workers (Folker, Chaikoff, Enteman & Tarver, 1951b) found, however, that conversion of methionine sulphur to cystine sulphur can take place in the eviscerated dog. An enzyme which is exclusively found in the liver is responsible for the conversion of phenylalanine to tyrosine (Udenfriend & Cooper, 1952). The enzyme complex catalysing the further oxidation of tyrosine is also mainly found in liver and it is thus possible that the liver plays a special role in the metabolism of aromatic and sulphur-containing amino acids.

2. Plasma-Protein Production

The proteins of the liver are in a state of dynamic equilibrium, i.e., they are subject to a continuous process of replacement which is independent of cell mitosis. This turnover—which affects all the subcellular fractions, the nucleus, the mitochondria, the microsomes and the cytoplasmic fluid, to varying extents—is, however, by no means peculiar to the liver; it occurs at greatly varying rates in all mammalian tissues. The special position of the liver in protein synthesis arises from the fact that the bulk of the plasma proteins is made in that organ. Miller and his colleagues have shown conclusively that in the rat all the albumin and fibrinogen and some of the α- and β-globulins are made in the liver, whilst other proteins of the α- and β-globulin group and all the γ-globulin are made elsewhere in the body (Miller, Bly, Watson & Bale, 1951; Miller & Bale, 1954; Miller, Bly & Bale, 1954). There is no reason to assume that this situation is peculiar to the rat; clinical evidence indeed strongly suggests that in man, too, the production of fibrinogen, albumin and some of the globulins depends on the proper functioning of the liver.

The actual amounts of the various proteins synthesized per day have been calculated either from experiments in which labelled amino acids or proteins were administered to normal subjects or from the results of investigations in which unlabelled proteins were injected into subjects in which a particular protein fraction was absent. With the exception of the observations on albumin (Bennhold, Peters & Roth, 1954), the results obtained by these two methods (Table I) show a fair measure of agreement. The difficulties in the interpretation of such experiments, which cannot be considered here in detail, have been discussed by various authors (McFarlane, 1952; Campbell, Cuthbertson, Matthews & McFarlane, 1956; Margen & Tarver, 1956). One of the main problems in a quantitative treatment arises from the fact that none of the electrophoretically defined fractions of plasma proteins, least of all the α- and β-globulins, is physically, and also probably metabolically, homogeneous. The half-life value calculated for a particular fraction is an over-all figure and may not apply to a quantitatively small, but functionally important, component of that fraction. Another difficulty is caused by the fact that a decrease of activity or concentration in the blood is due not only to catabolism but also to the mixing of the protein in the plasma with extravascular protein. The amount of plasma protein outside the circulation has been calculated to be about one-and-a-half times greater than that found in the blood; moreover, it is likely that this exchange will not take place at a uniform rate. Little is known about the factors controlling the rate of plasma-protein production; nor do we know how far this rate can be varied in response to metabolic needs.

TABLE I. HALF-LIFE VALUES OF VARIOUS PLASMA PROTEINS IN MAN, DETERMINED BY ISOTOPE AND BIOLOGICAL METHODS

Protein	Half-life (days)	
	Isotope method	Biological method
Albumin .	26[a]	70[b]
α-Globulin .	8-10[c]	10[d]
γ-Globulin .	20-30[d]	35[e]
Fibrinogen .	2-3[f]	2-3[g]

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In a variety of liver conditions a large reduction of serum albumin concentration in the blood may be found. This could be caused in three ways: (i) The total amount present in the body may be unchanged, but a larger proportion than in the normal is in the extravascular compartment; this might be caused by a change in capillary permeability or by an expansion of extracellular space. (ii) The decrease might be due to an increased rate of catabolism, the rate of production being within normal limits. (iii) The rate of production is decreased, with the rate of breakdown being unchanged or only slightly reduced. It is generally assumed, often without convincing evidence, that it is the last cause which is responsible for any lowering of serum albumin concentration which we may find in liver disease. This belief is probably correct in most cases, but it would be wrong to assume that the albumin level gives a quantitative measure of the rate of albumin production at the time the sample is taken. In the first place, it is possible that the body may partly compensate for a decrease in the rate of albumin synthesis by a lowering of the rate of catabolism. Secondly, the decrease in concen-

tration will be apparent only some time after the liver lesion has occurred. Thus, Margen & Tarver (1956) have calculated that there are about 500 g. of serum albumin in the body of the normal adult, about a third of which is in the blood. With a half-life of 26 days, the daily production of albumin is approximately 12 g. Assuming that the rate of catabolism does not change, it will take 16 days before a 50% reduction in the rate of albumin synthesis leads to a decrease of 20% in serum albumin concentration. Even a complete cessation of albumin production will result only in a 20% decrease of blood level after about eight days. If the rate of catabolism should fall in response to a decrease in concentration, plasma concentration will decrease even more slowly. It thus appears that the level of albumin concentration reflects the functional state of the liver at an earlier period and is, possibly, altogether an unreliable measure of the rate of albumin synthesis. This is probably the reason why the serum albumin values are generally normal in acute conditions such as hepatic necrosis and are often normal in chronic hepatitis, and also why liver function tests which depend on the relative concentrations of the different plasma proteins still give abnormal results some time after clinical recovery has taken place.

B. SPECIAL PROBLEMS ASSOCIATED WITH LIVER DISEASE

N. H. MARTIN

3. The Relation of Protein Intake to Liver Damage

a. Experimentally Induced Starvation

Up to 1939 perhaps the best-known data on the results of diminished food intake on man were the Carnegie experiments carried out by Benedict (1915). In view of the wide publicity this work has received it is worth recalling that the observed weight loss was only about 10%, and that, though ahead of their time, by modern standards the experiments left something to be desired.

At the outset of the 1939–45 war we were surprisingly ignorant of the physical and psychological consequences of semi-starvation. To gather exact information on the matter Ancel Keys and his collaborators (Keys, Brozek, Henschel, Mickelsen & Taylor, 1950) carried out a precisely controlled investigation on a group of healthy volunteers in Minnesota. In the Minnesota experiment a starvation period of 24 weeks produced a weight loss of the order of 25%. The group was maintained on a diet of 1,500 calories a day for the whole period, the diet containing a total of 50.8 g. of protein of which 7.3 g. were derived from meat or milk. Leyton (1946), at Tost in Germany, had observed a 20% weight loss among allied prisoners after semi-starvation on a diet of about 1,600 calories, containing 55 g. of protein, of which 5 g. were derived from meat, for a period of about six months, while Walters, Rossiter & Lehmann (1947) had observed a 25% weight loss in semi-starving Indian prisoners in the Far East. Keys's controlled experiments do, therefore, appear to reproduce levels of inanition resulting from naturally occurring semi-starvation. The Minnesota experiments were not designed specifically to disclose derangement of the liver in the volunteers, but there seems little doubt that, had any significant derangement occurred, Keys and his colleagues would have observed it. In Keys's group Z, during the period of semi-starvation the mean haemoglobin dropped from 15.0 g. to 11.0 g./100 ml. and the total plasma protein dropped from 6.5 g. to 6.05 g./100 ml. The ratio of albumin to the globulin did not alter significantly, nor did the individual globulin fractions alter. Although liver biopsy was not performed, there was no clinical or biochemical evidence of liver damage. The group showed some dependent oedema.

Beattie, Herbert & Bell (1948), in their observations on groups of semi-starved persons under medical care following the liberation of Holland, described much the same general picture. In *Studies of undernutrition, Wuppertal 1946–9*, by McCance *et al.* (see Medical Research Council, 1951), full electrophoresis was carried out on a carefully selected series, which confirmed that in uncomplicated starvation, while there was some diminution in the level of total circulating protein, there was no significant change in the pattern of the individual components. There was no biochemical evidence of liver insufficiency in the Wuppertal series, nor did the hepatic biopsy material obtained show histological evidence suggestive of liver damage, though there was an increase in iron-containing pigment. The most consistent feature at autopsy, in cases of pure starvation or semi-starvation, is the loss of weight of the liver. Where data are available, the relative loss of weight by the liver is shown to be greater than by the whole body (Ühlinger, 1948).

b. Protein Depletion in the Tropics

It is against the background of these experiments and field studies that one needs to examine the accumulated observations on tropical malnutrition which is so often associated with unequivocal evidence of liver damage (Waterlow, 1948; Davies, 1948; Walters & Waterlow, 1954). Studies of infant nutrition in the tropics in the last two decades have laid increasing stress on the relation between the quantity and the quality of the dietary protein and the integrity of the liver (Dean, 1952; Thomson & Duncan, 1954). Diets determined by custom and economic circumstance, unlike those devised for clinical investigation, are rarely grossly deficient in protein without at the same time lacking some accessory food factors. Natural starvation therefore presents a varied clinical picture and responds irregularly to specific therapy. In addition, the field worker in the tropics, when selecting subjects for exhaustive study, is faced with the frequency of endemic parasitic infection and the difficulty of obtaining precise information on the amount and type of food taken, except over a limited period. Even if such information is obtained, the problem remains of introducing satisfactorily adjusted diets and maintaining such diets once the patient has left hospital, thus making a real assessment of clinical and biochemical improvement difficult. Children have been the principal subject of study, though investigations on adults have been reported (Trowell, 1949). The patients are frequently oedematous and have swollen bellies; their haemoglobin levels are below normal, as are the serum total protein levels, with a significant relative diminution in the level of albumin. Biopsy and autopsy material generally shows either increased infiltration with fat or a fine periportal or diffuse fibrosis. The increased amount of iron-containing pigment emphasized by Gillman & Gillman (1948) is not a regularly associated finding. Figures available for adults suggest that the diet of

such patients has rarely exceeded 2,000 calories a day, 80% of this being derived from carbohydrate. The daily protein intake rarely exceeds 50 g., of which perhaps 5–10 g. are of animal origin. The dietary background therefore resembles that of the volunteers in the Minnesota experiment in its calories and protein intake. Parasitic infection and more particularly malarial infestation are so common in many of the tropical areas where studies have been made that it is impossible to judge to what extent these might be factors in producing the striking changes observed in the liver.

It must be accepted that experiments and field studies under carefully controlled conditions in temperate climates give no support to the view that pure protein depletion of the adult for periods up to six months produces demonstrable chemical or histological lesions in the liver. However, adults in the tropics are not comparable with the adults studied in the laboratory and field in temperate zones, because they must be considered as survivors of a continuous environment of malnutrition, whereas the undernutrition associated with protein depletion in the experimental groups represents a very short period in terms of total life-span. Such an argument leaves the part played by chronic infection unresolved. Thus field studies in the Ruanda-Urundi suggest that among the Batussi, a pastoral people and cattle breeders, whose children drink milk freely, infant liver damage is exceptional, whereas among the agricultural Bahutu, who come from the same area but are not herdsmen, infant liver damage is common (Brock & Autret, 1952). It is reasonable to assume that these two tribes are subject to the same general endemic infections, and that the only substantial difference is the dietary habit which persists from infancy.

c. Arrest of Established Liver Damage

In spite of the brilliant experimental work on rats and other animals there is no clear-cut evidence that choline, inositol, tocopherol, or methionine will arrest or improve liver damage contracted and established in the natural state in man, though improvement is claimed after dietary supplementation with milk proteins, liver extracts and extracts of hog's stomach. Relevant data may be found in the papers of Lindan & Himsworth (1950); György (1951); Kosterlitz (1954); Hartroft (1954); and Best, Lucas & Ridout (1954).

4. Liver Damage and Circulating Proteins

a. Changes in the Levels of Plasma Proteins, Associated with Liver Damage

Alterations in the levels of the serum proteins of patients suffering from all forms of liver damage have been studied repeatedly ever since Abrami & Wallick (1929) in France, and Salvesen (1929) in Scandinavia, demonstrated the marked alteration that may occur in the relative levels of the serum albumin and globulins of patients suffering from chronic liver damage. In the last decades the application of electrophoretic techniques to the analysis of serum proteins has enabled a more detailed picture of the serum protein pattern to be obtained through the various stages of liver damage, from the acute phases of infective and serum-transmitted hepatitis (Gray & Barron, 1943; Martin, 1946) to the established chronic hepatitis with fibrotic changes in the substance of the liver (Martin, 1949a; Ricketts & Sterling, 1949). The changes resulting from biliary cirrhosis have been studied specifically (Sterling & Ricketts, 1949). Superficial inspection of these studies suggests that in the acute phase of hepatitis there is a relative decrease in the level of serum albumin, the globulins remaining relatively constant. As the disease progresses there may be some disturbance of the γ- and β-globulin levels, but this is neither constant nor regular. If the damage persists beyond the fourth to sixth week there is a tendency for the γ-globulin to rise. In the majority of patients the changes are not marked and frequently the values lie between the normal limits. In uncomplicated chronic progressive liver damage the disturbance of pattern is more striking, there being a reversal of the albumin:globulin ratio with a marked relative increase in the concentration of the globulins. Electrophoretic analysis shows a γ-globulin peak with a wide base very different from that seen in such conditions as myelomatosis, in which electrophoresis commonly shows a striking increase in the γ-globulins. The serum protein derangement observed in cirrhosis appears to be independent of aetiological background; it is not pathognomonic of the condition, nor is it found in every case.

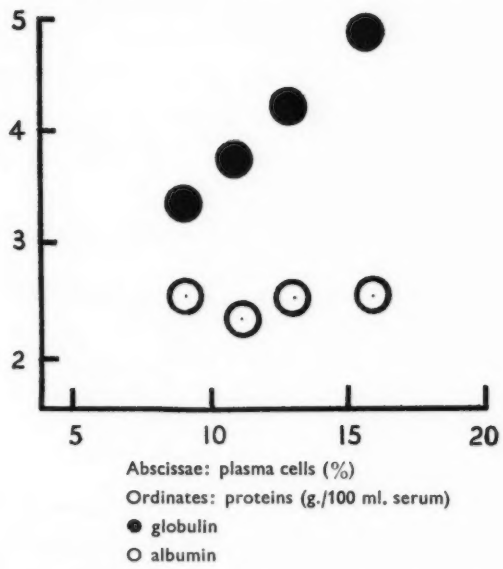
b. Alterations in Total Amounts of Circulating Proteins

The studies of Labby & Hoagland (1947) on the movements of body-fluids in acute liver disease suggest that some of the fluctuations in protein level may be due to alterations in plasma volume. It also seems possible that the apparent alterations in albumin:globulin ratio noted in early stages of the disease represent an increased output of globulin rather than a diminished production of albumin. The production of human albumin, in sufficient quantities to make replacement therapy analogous to blood transfusion possible in conditions believed to be associated with a diminution of circulating albumin, focused attention on the total circulating proteins rather than the relative concentrations. While studying a group of 30 patients with liver damage receiving intravenous albumin, Martin & Davies (1955) uncovered eight patients in whom there was unequivocal evidence of an absolute increase in circulating globulin. The increase, which ranged up to 242 g. of circulating globulin, was due to a gross excess of γ-globulin. Hiller, Huffman & Levey (1949) in the USA, studying 12 patients with liver damage, had arrived at a mean value for circulating globulin of 137 g. compared with a mean of 79.1 g. for 17 normal American males. Jarrold & Vilter (1949), in detailed examinations of the bone-marrow from 30 patients with established liver damage, observed a substantial increase in plasma cells in 16. If due allowance be made for inevitable sampling errors, counts of 15% and above must be regarded as abnormal. These authors produced data, moreover, suggesting a correlation between plasma-cell counts and globulin levels. A plot of existing data (fig. 1) illustrates this correlation and suggests that there is no correlation with albumin levels. In chronic hepatitis hyperglobulinaemia is a finding that deserves study independent of albumin levels.

c. Alterations in Circulating and Tissue Enzymes

If it be assumed that certain enzymes found in plasma are elaborated in the liver, studies of their qualitative and quantitative behaviour might be a fruitful field for investigation of diseases of the liver. It is possible also that detailed examination of the enzyme disturbance might open an approach to the study of the effect of liver damage on protein structure.

FIG. 1. CORRELATION OF PLASMA-CELL COUNTS IN BONE-MARROW AGAINST SERUM PROTEIN LEVELS



McArdle (1940) demonstrated that in established liver disease the activity of the pseudocholinesterase in the serum was lower than normal, and the question arose whether qualitative changes in the enzyme occurred also. Surgenor (1949) using cold ethanol fractionation, and Kekwick, Mackay & Martin (1953) using cold ether fractionation, have achieved a considerable concentration of this enzyme; from their work this appears to be associated with the α-globulins in human serum. Kekwick (1955), in a small group of patients, was able to make a detailed study of the characteristics of the enzyme in liver damage, using as his criterion a multiple substrate pattern. Adams & Whittaker (1949) had demonstrated that in normal human serum the optimal acyl group for hydrolysis at pH 7.4 was butyryl, and Kekwick (1955) was able to confirm this. However, the esterase of patients suffering from long-established damage appeared to have a distorted pattern, the optimal acyl group under similar conditions being propionyl. (See fig. 2.) Though the simplest explanation of this observation is that advanced liver damage has affected a closely related group of enzymes differentially, it is possible that the distortion of substrate behaviour pattern represents a distortion of the configuration of a single enzyme.

Limited studies available (Waterlow, 1952) indicate that the concentrations of pseudocholinesterases in liver tissue run parallel with their fluctuations in serum, in spite of the fact that other tissues in the body are known to be rich in pseudocholinesterase. This is in line with the comparative studies of Sherlock & Walshe (1947) on the alkaline phosphatase fluctuations associated with liver damage, even though these authors at that time felt that their studies supported the concept of an extra-hepatic origin for the alkaline phosphatase they studied.

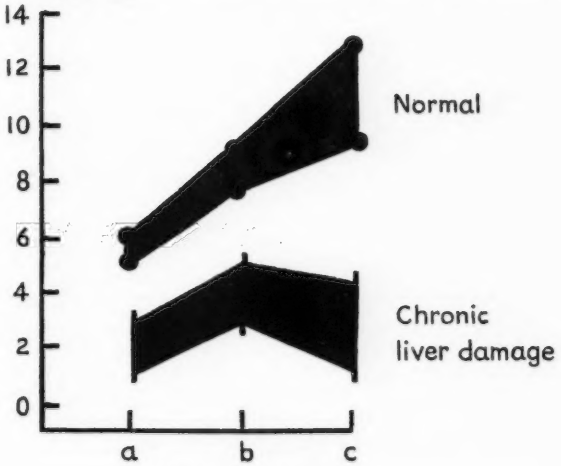
Attention has recently been focused on the changes in serum transaminase in cardiac ischaemia. Numerous articles have appeared indicating that, unlike esterase activity, there is a marked increase in serum transaminase activity of patients with liver damage (Wróblewski & LaDue, 1955; Chinsky, Shmagranoff & Sherry, 1956) but, as the enzyme is wide spread in the body, the significance of these findings is not clear. Recent detailed study of the behaviour of serum transaminase in acute liver damage suggests, however, an intimate relationship between serum levels and liver integrity (Karmen, Wróblewski & LaDue, 1955; Wróblewski, Jervis & LaDue, 1956).

d. Qualitative Changes in Circulating Proteins, Associated with Liver Damage

Charlwood (1954), in a series of physico-chemical studies of albumin separated by electrophoresis from the sera of a series of patients suffering from liver damage, observed no unequivocal difference in their sedimentation value compared with values for normal albumin examined under the same conditions, nor in the calculated value of their diffusion constant, nor do such data as have been obtained on electrophoretic mobility indicate a significant difference from normal values. It must be assumed, therefore, that the disease produces no significant difference in net charge, nor in mean molecular weight. Physico-chemical measurements such as these, however, deal only with statistical means; they are "average" measurements and do not exclude the possibility of more minute configurational differences.

For many years empirical flocculation tests have been used, with some advantage, in the clinico-chemical assessment of liver damage. Maclagan (1948) and Maclagan, Martin & Lunnon (1952) have reviewed the data on the mechanism of these tests. Though a number of the tests seem to be correlated

FIG. 2. SERUM-PSEUDOCHOLINESTERASE ACTIVITY PATTERNS FROM PERSONS IN NORMAL HEALTH AND FROM PATIENTS WITH ADVANCED LIVER DAMAGE



with the levels of circulating γ-globulin, two, the thymol and the cephalin cholesterol tests, certainly depend upon additional factors. It appears that albumin separated from patients with liver damage differs from that separated from normal persons in its capacity to inhibit the thymol flocculation test. Martin (1948, 1949b) and Martin & Perkins (1954) have suggested that the difference may be in the pattern of charge on the surface of the albumin molecule. But, in view of the data of Charlwood (1954), more precise evidence is needed before this suggestion can be accepted.

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THE LIVER AND STEROID HORMONE METABOLISM

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1 Adrenocortical hormones
a Hydrocortisone
b Aldosterone
2 Progesterone
3 Testosterone
4 Oestrogens
5 The conjugating function of the liver
6 Clinical aspects
References

The continuing interest of a large number of research workers in the metabolism of the steroid hormones is rapidly increasing our understanding of some aspects of liver function. Advances have been hastened by the development of more accurate and subtle methods of analysis, such as the application to steroid biochemistry of paper chromatography by Bush (1951)[1] and Zaffaroni & Burton (1951), the use of ^{14}C-labelled steroids, and refinements of classical chemical procedures, e.g., the method of Brown (1955) for the estimation of certain oestrogens.

This report aims at summarizing the conclusions that are emerging and the evidence on which they are based. Considerations of space allow mention of only some of the workers who have contributed. Because the metabolism of steroid hormones varies widely in different species the emphasis will be placed on human metabolism, and all evidence cited refers to human material unless otherwise stated. More consideration has been given to in-vivo than to in-vitro experiments, as the extent to which the two are related is often uncertain.

[1] See also Bush, *Brit. med. Bull.* 1954, **10**, 229.—Ed.

1. Adrenocortical Hormones

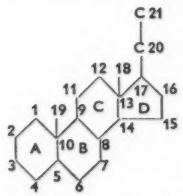
Bush & Sandberg (1953) have shown that the major free adrenocortical steroid in human plasma is hydrocortisone (I), with corticosterone present in considerably smaller quantities. Relatively minute quantities of a number of other steroids have been detected in adrenal venous blood, and one with marked salt-retaining properties, aldosterone (II), has been isolated and its formula elucidated (Simpson, Tait & Bush, 1952; Simpson, Tait, Wettstein, Neher, Euw, Schindler & Reichstein, 1954). Hydrocortisone and aldosterone together appear to be able to reproduce all the effects of adrenocortical stimulation and, although not necessarily the only important adrenocortical hormones, are the only ones whose metabolism will be considered here.

a. Hydrocortisone

When hydrocortisone is infused intravenously into man, an initially high plasma hydrocortisone concentration rapidly falls. A clearer understanding of what happens to the infused steroid (and, by analogy, to endogenous hydrocortisone) emerges from the findings of Hellman, Bradlow, Adesman, Fukushima, Kulp & Gallagher (1954); Sayers, Glenn, Sydnor, Lipscomb, Sweat, Kelly, Levy & Jeffries (1955); Peterson, Wyngaarden, Guerra, Brodie & Bunim (1955). Giving [4-^{14}C]hydrocortisone intravenously, Hellman *et al.* (1954) found that the proportional rate of disappearance from plasma, and the rate of excretion in urine of isotopically labelled steroid, were the same whether large (100 mg.) or small (0.25 mg.) amounts of steroid were infused. The pattern of urinary excretion after hydrocortisone infusion was the

A SHORT GLOSSARY OF CHEMICAL SYMBOLS AND NAMES USED IN THIS PAPER

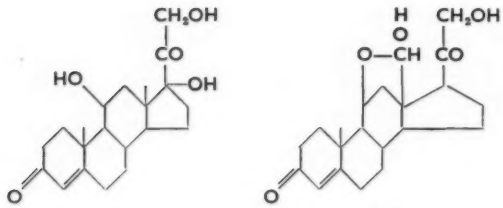
As all readers may not be familiar with certain conventions of chemical nomenclature, a short list of symbols and chemical names used in the text is given below.



Steroid nucleus showing conventional numbering of carbon atoms

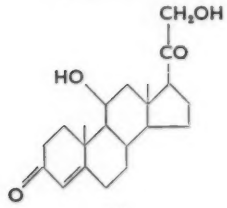
4-C or C-4	the number 4 carbon atom
^{14}C	radioactive isotope of carbon, atomic weight 14
Δ^4	signifies a double bond between C-4 and C-5
-ol	suffix indicating —OH group
-one	suffix indicating =O group
α and β	used to describe whether an —OH group or —H atom lies above the plane of the molecule (β)—, or below (α)—

hydrocortisone	Δ^4-pregnene-11β:17α:21-triol-3:20-dione
corticosterone	Δ^4-pregnene-11β:21-diol-3:20-dione
tetrahydrocortisone	pregnane-3α:17α:21-triol-11:20-dione
aldosterone	Δ^4-pregnene-11β:21-diol-3:20-dione-18-al
11β-hydroxy-aetiocholanolone	aetiocholane-3α:11β-diol-17-one
testosterone	Δ^4-androstene-17β-ol-3-one
androsterone	androstane-3α-ol-17-one
aetiocholanolone	aetiocholane-3α-ol-17-one
androstenedione	Δ^4-androstene-3:17-dione
progesterone	Δ^4-pregnene-3:20-dione
pregnanediol	pregnane-3α:20α-diol

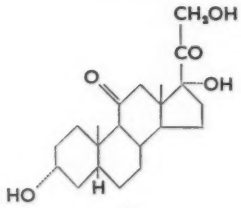


I
Hydrocortisone

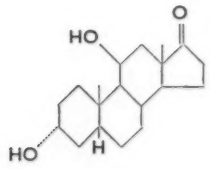
II
Aldosterone



III
Corticosterone



IV
Tetrahydrocortisone



V
11β-Hydroxyaetiocholanolone

same whether the tissues were depleted of endogenous steroid or saturated with prior large dosage of exogenous hydrocortisone, suggesting that widely differing amounts of hormone are handled in a similar fashion and that the mechanism is independent of any functional utilization. Very similar results were obtained by Sayers *et al.* (1955) using unlabelled hydrocortisone. They confirmed that the pattern of disappearance of large amounts (100 mg.) of infused hydrocortisone was independent of functional requirements of the tissues. They followed hydrocortisone concentrations in plasma at intervals and calculated that, at the end of the 30-minute infusion, the blood contained no more than 5% of the infused hydrocortisone as unchanged hydrocortisone. Half of this again was altered or removed from the circulation in the next 1.3–2.3 hours. Disappearance curves suggested that at least two processes were involved in the removal of hydrocortisone from the circulation. Peterson *et al.* (1955) in similar experiments reached the same conclusions except that they interpreted their disappearance curves (of plasma hydrocortisone) as more characteristic of a first-order reaction.

The importance of the liver in this removal of hydrocortisone from the circulation is emphasized by recent work. The finding that the peak excretion of hydrocortisone and/or metabolites does not occur at once, but is delayed until the second hour after intravenous infusion (Hellman *et al.* 1954), indicates that the hormone has been involved in some intermediate process before it is excreted in the urine. Direct renal clearance of the unaltered hormone accounts for less than 1% of administered hydrocortisone (Peterson *et al.* 1955), which can only have diffused into extracellular fluid or been taken up in cells. Bradlow, Dobriner & Gallagher (1954) found in mice that 70% of a tritium-labelled dose of cortisone given intravenously could be found in the liver within five minutes of administration, and it seems likely that something similar occurs in man from the work of Plager, Tyler, Hecht & Samuels (1954) who were able to demonstrate reduced radioactivity in human hepatic venous blood, compared with simultaneous arterial blood radioactivity after intravenous infusion of [4-^{14}C]hydrocortisone. Further indirect evidence has been obtained from patients with chronic liver disease. Plasma levels of 17-hydroxycorticosteroids in patients with liver disease are normal, and the rise after corticotrophin infusion is also within normal limits (Brown, Willardson, Samuels & Tyler, 1954). However, infused hydrocortisone disappears from the circulation at a slower rate than normal (Brown *et al.* 1954; Peterson *et al.* 1955), although the rate of disappearance of the related steroids cortisone, corticosterone (III), tetrahydrocortisone (IV), and others (which all normally disappear at rates faster than hydrocortisone) is much the same in healthy as in cirrhotic subjects (Peterson *et al.* 1955). These findings in patients with cirrhosis of the liver suggest that hydrocortisone is metabolized by a highly specific process which can deal with physiological amounts of hydrocortisone even in the presence of liver disease; but, whereas in the healthy liver this pathway can carry large amounts of infused hydrocortisone, the damaged liver has too small a reserve capacity to do likewise.

From 80% to 90% of hydrocortisone metabolites are excreted in the urine (Hellman *et al.* 1954). Details of what happens to hydrocortisone in the liver cell have been sought by many workers in the study of these urinary metabolites. Although classical isolation and characterization techniques have been used infrequently in these studies, there is good evidence that the metabolic change involves reduction of the steroid nucleus and its substituents, with or without subsequent splitting of the side-chain (Dorfman & Ungar, 1953).

The reduction of ring A of the steroid molecule occurs in two stages. The first involves reduction of the 4:5-unsaturated group and is apparently irreversible in vivo (Savard & Goldfaden, 1954; Ungar, Davis, Rosencrantz & Dorfman, 1954). Dorfman and his colleagues (see Dorfman & Ungar, 1953) have emphasized the predominance of reduced derivatives possessing the pregnane (5β) configuration, and they adduced evidence that this preferred steric form is induced by the side-chain at C-17. The second stage is the reduction of the 3-ketone group to 3α-hydroxyl, and this conversion is reversible in vivo (Savard & Goldfaden, 1954). The steroids with reduced ring A, which are biologically inactive, are excreted in the urine conjugated with glucuronic acid, and form a major fraction of the metabolites of hydrocortisone (Schneider, 1950; de Courcy, Bush, Gray & Lunnon, 1953; Burstein, Savard & Dorfman, 1953). This reduction in ring A appears generally to precede changes in the side-chain at C-17 (see Dorfman & Ungar, 1953). However, de Courcy *et al.* (1953) have provisionally identified a urinary steroid, presumably derived from hydrocortisone, with reduced oxygen function at C-20 and still retaining an unreduced Δ^4-3-ketone group in ring A.

Removal of the side-chain from the hydrocortisone molecule leaves a 17-ketone group, and the principal 17-ketosteroids excreted after hydrocortisone administration retain their oxygen function at C-11 and have the 5β-configuration (aetiocholane series). 11β-Hydroxyaetiocholanolone (V) is

the most abundant 17-ketosteroid metabolite, there being rather less of the 11-keto analogue, and removal of the oxygen function at C-11 does not occur (Burstein *et al.* 1953). It should be remarked, however, that many of these findings relate to studies in a few individuals, and the extent to which they apply to the human species as a whole is not certain. Other workers have isolated 17-ketosteroids with the 5α-configuration (androstane series) in the urine of patients receiving hydrocortisone (Gallagher, 1954; Kemp, Kappas, Salamon, Herling & Gallagher, 1954).

Nothing is known of the chemical nature of any non-steroid metabolites of hydrocortisone. Radioactivity of expired air after administration of [4-^{14}C]hydrocortisone is negligible (Hellman *et al.* 1954), which suggests that complete disruption of the steroid molecule occurs on only a small scale if at all.

All the preceding discussion has been concerned with the results of in-vivo experiments. There has been a considerable amount of recent work on the metabolism of hydrocortisone in vitro, sometimes with results that conflict strikingly with the findings of the in-vivo studies.

Using mainly rat liver preparations, many groups of workers have convincingly demonstrated the capacity of liver to reduce the Δ^4-3-ketone group, to reduce the C-20-ketone to C-20-hydroxyl, and to split off the side-chain at C-17. In contrast to the findings in in-vivo experiments, reduction at C-5 results exclusively in steroids possessing the *allo*pregnane (5α-)configuration. Because the relationship of these experiments to in-vivo metabolism in man is doubtful, they will not be discussed more fully here. A comprehensive review by Roberts & Szego (1955a) covers this aspect of steroid metabolism.

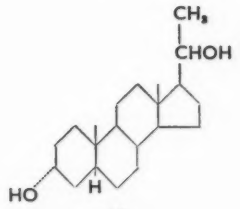
Faecal excretion of hydrocortisone metabolites is slight in man (Hellman *et al.* 1954; Plager *et al.* 1954), less than 10% of radioactivity appearing in the faeces after administration of [4-^{14}C]hydrocortisone. This is again in marked contrast to the finding in rodents and emphasizes the need for caution in drawing conclusions in human physiology from rodent experiments.

b. Aldosterone

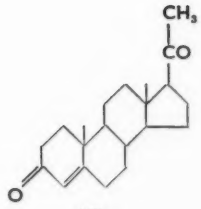
Because of the low concentration of this steroid in biological fluids, with consequent difficulties in estimation, and because large amounts are not available for the types of in-vivo experiment that have been utilized in studying hydrocortisone metabolism, direct knowledge of aldosterone metabolism is almost non-existent. It seems very likely that the liver is intimately concerned in its inactivation, both from the similarity of its structure to hydrocortisone, and from the work of Chart, Gordon, Helmer & Le Sher (1956) who incubated an active salt-retaining fraction, isolated from human urine, with human liver slices. Surviving liver slices brought about complete or nearly complete loss of sodium-retaining activity, whereas boiled liver slices were without effect.

2. Progesterone

Since Marrian (1929) isolated pregnanediol (VI) from the urine of pregnant women, the reduction of progesterone (VII) in part to pregnanediol has been well documented. Administration of progesterone increases the urinary excretion of pregnanediol (Venning & Browne, 1937) and *allo*pregnanediol (Kyle & Marrian, 1951). Reports that the presence of a secretory endometrium influences this conversion in vivo have not been substantiated (see Klopper & Michie, 1956), and



VI
Pregnanediol



VII
Progesterone

attention has been directed mainly towards the liver. Wiswell & Samuels (1953), using homogenates of rat and rabbit liver, have demonstrated reduction of the Δ^4-3-ketone group in ring A of incubated progesterone. No 17-ketosteroids were demonstrable in the medium after incubation, indicating that no oxidative removal of the side-chain at C-17 had occurred. Participation by other tissues in the ring A reductive process has not been ruled out.

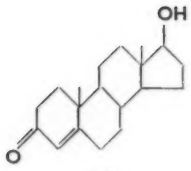
Whether or not it is exclusively formed in the liver, pregnanediol is certainly excreted in the bile (Rogers & McLellan, 1951). Wiest, Fujimoto & Sandberg (1955) found that, after giving [4-^{14}C]progesterone intravenously to a woman with a biliary fistula but with normal liver function, there was twice as much radioactivity in bile as in urine in the first 24 hours after injection. In four out of six patients with biliary obstruction given large oral doses of progesterone, Rogers (1956) found a urinary pregnanediol excretion higher than normal, which fell when the obstruction was relieved. Gallagher, Bradlow, Fukushima, Beer, Kritchevsky, Stokem, Eidinoff, Hellman & Dobriner (1954) reported that, after giving [4-^{14}C]progesterone to two normal subjects, about 30% of the administered radioactivity appeared in the urine. Excretion in bile is therefore an important pathway for progesterone metabolites, although the extent to which reabsorption from the intestine occurs is still uncertain.

Reduction of the Δ^4-3-ketone group in ring A of progesterone results mainly in derivatives of the pregnane (5β) series. Kyle & Marrian (1951) found the ratio of pregnanediol to *allo*pregnanediol in urine after administration of progesterone to be 30:1. Other *allo*pregnane (5α) derivatives have been isolated from human pregnancy urine (Salamon & Dobriner, 1954; Lieberman, Praetz, Humphries & Dobriner, 1953), but they may not be derived from progesterone.

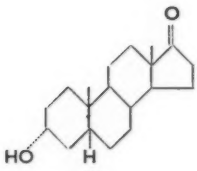
Data on urinary progesterone metabolites in liver disease are scanty and difficult to interpret. Paschkis, Cantarow & Havens (1951), investigating patients with severe hepatic disease receiving 100 mg. progesterone intramuscularly daily, reported that pregnanediol excretion appeared to be higher than normal. In 12 similar patients receiving large doses of progesterone orally, Rogers (1956) found urinary pregnanediol excretion normal in four, below normal in six, and above normal in two. No consistent clinical abnormality distinguished these groups.

3. Testosterone

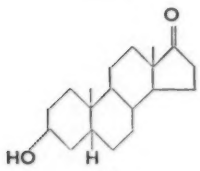
Isotopically labelled testosterone (VIII) has been used to give an over-all picture of testosterone metabolism. After slow intravenous infusion of deutero-testosterone and [4-^{14}C]deutero-testosterone into two men, Fukushima, Bradlow, Dobriner & Gallagher (1954) found that all the testosterone rapidly disappeared and its metabolites were soon excreted



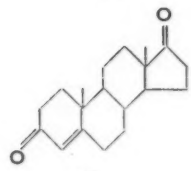
VIII
Testosterone



IX
Androsterone



X
Aetiocholanolone



XI
Androstenedione

in the urine. Of the infused radioactivity 30% appeared in the urine during the 5-hour infusion period. Urinary excretion over the first 48 hours was about 60% of the administered radioactivity. Faecal excretion accounted at most for a further 10–15%. No significant radioactivity was detected in the respired air, but dilution factors are so great that this does not exclude significant oxidation of steroid to carbon dioxide.

The bulk of evidence linking the liver with testosterone metabolism has been derived from rodents and other laboratory animals. In these, testosterone metabolites are excreted mainly into the bile, and the greater part of any administered radioactivity appears in the faeces (Barry, Eidinoff, Dobriner & Gallagher, 1952). This marked difference from the pattern of distribution in human subjects means that results of such experiments cannot readily be extended to explain human metabolism of testosterone. In-vitro investigations of liver function with human rather than rodent material have been scanty. Samuels, McCaulay & Sellers (1947) showed that human liver mince, like rodent liver mince, is capable of destroying testosterone with reduction of the Δ^4-3-ketone group. However, as they found in the incubation mixture no 17-ketosteroids, which are the predominant metabolites of testosterone in vivo, the relevance of these in-vitro findings is not certain.

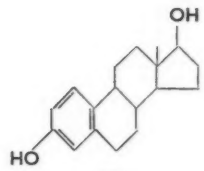
The principal urinary metabolites of testosterone have long been recognized as androsterone (IX) and aetiocholanolone (X) (Callow, 1939), accounting for 90% of the urinary radioactivity present after the administration of labelled testosterone (Fukushima *et al.* 1954). The conversion of testosterone to androsterone and aetiocholanolone proceeds in two stages (see Dorfman & Ungar, 1953). The first, oxidation of the C-17 hydroxyl to a ketone group, is accomplished by a wide variety of human tissues such as skin, intestine, smooth muscle, myometrium, breast, and various tumours (Wotiz, Lemon & Voulgaropoulos, 1954), as well as liver (Samuels *et al.* 1947). Wotiz *et al.* (1954) reported that the most abundant metabolite after incubation of testosterone with tissues other than liver was androstenedione (XI), which is not normally excreted in the urine and must undergo reduction of its Δ^4-3-ketone function. This, the second stage in the conversion of testosterone to saturated 17-ketosteroid derivatives, can be brought about by liver tissue (Samuels *et al.* 1947), but participation by other human tissues has not been ruled out.

In-vivo studies of patients with liver disease have been conflicting. Thus, West, Tyler, Brown & Samuels (1951) interpreted their data obtained from infusion of testosterone into cirrhotic and healthy subjects as indicating that patients with liver disease have a decreased ability to convert testosterone to 17-ketosteroids. This was not the finding of Williams, Cantarow, Paschkis & Havens (1951) who reported that after intramuscular injection of testosterone the excretion rate of urinary 17-ketosteroids in cirrhotic patients was very similar to that in healthy controls. I have been impressed with the ability of many patients with severe liver disease to respond to large intravenous infusions of testosterone by prompt excretion of large amounts of 17-ketosteroids in the urine (unpublished observations).

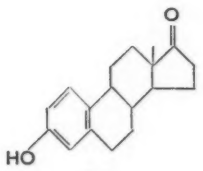
It can be said, therefore, that an important metabolic and excretory function has been demonstrated for the liver of rodents and some other animals; but in human liver the excretory function appears to be minimal, and any specific metabolic function has not yet been conclusively demonstrated.

4. Oestrogens

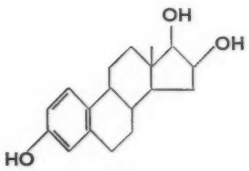
The primary hormones discussed so far have been clearly established as such, but the primary form of the oestrogenic hormone is still undecided. Both oestradiol (XII) (MacCorquodale, Thayer & Doisy, 1936) and oestrone (XIII) (Westerfield, Thayer, MacCorquodale & Doisy, 1938a) have been isolated from sow ovary. In human placenta there is oestrone (Westerfield, Thayer, MacCorquodale & Doisy, 1938b), oestradiol (Huffman, Thayer & Doisy, 1940) and



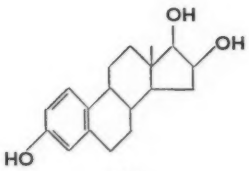
XII
Oestradiol



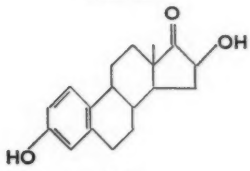
XIII
Oestrone



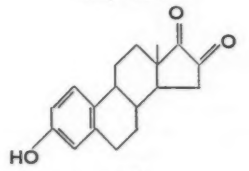
XIV
Oestriol



XV
16-epiOestriol



XVI
16α-Hydroxyoestrone



XVII
16-Keto-oestrone

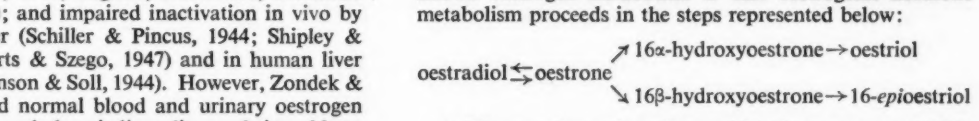
oestriol (XIV) (Browne, cited by Collip, 1930), and all occur in relatively high concentrations (Diczfalusy, 1953; Mitchell & Davies, 1954). Oestradiol has been provisionally identified in human testis (Goldzieher & Roberts, 1952). Until any oestrogen present in the venous effluent from these organs is identified, the nature of the primary oestrogenic hormone is uncertain.

Since Zondek (1934) reported the rapid disappearance of injected oestrogen from the carcasses of rats, many workers have investigated liver function in oestrogen metabolism. Golden & Severinghaus (1938) and Segaloff (1943) have reported reduced physiological activity of oestrogens implanted within the drainage area of the portal tract of rodents, but Hooker, Drill & Pfeiffer (1947) were unable to demonstrate this effect in the monkey. There have been reports of inactivation of oestrogen in vitro by human liver preparations (Twombley & Taylor, 1942; Tagnon, Lieberman, Schulman & Brunschwig, 1952); and impaired inactivation in vivo by damaged animal liver (Schiller & Pincus, 1944; Shipley & György, 1944; Roberts & Szego, 1947) and in human liver disease (Glass, Edmonson & Soll, 1944). However, Zondek & Black (1947) reported normal blood and urinary oestrogen levels in severe acute and chronic liver disease, being able to demonstrate an alteration in oestrone metabolism only in moribund patients. Oestrogens have been demonstrated in human bile (Cantarow, Rakoff, Paschkis, Hansen & Walking, 1943; Pearlman & Rakoff, 1949). Despite all this evidence linking oestrogen metabolism and the liver in a general way, understanding of specific details of oestrogen metabolism, and even of what is meant by "inactivation", is far from clear.

The reason for this is that until very recently methods for the chemical identification and measurement of individual oestrogens have not been sufficiently accurate for the small concentrations in biological fluids. This has led to almost complete reliance on bio-assay methods, usually the Allen-Doisy test which is very sensitive to oestradiol and considerably less so to oestrone and oestriol. Conversion of oestradiol to oestrone or oestriol would be reflected therefore in loss of activity in the Allen-Doisy test. However, although it is difficult to determine the relative potencies of the various endogenous oestrogens under in-vivo conditions in human beings, there is much to suggest that in man the activity of oestrone, oestradiol and oestriol is not dissimilar. A further complication is lack of knowledge of the effect that conjugation of the steroid molecule has on biological activity. It is fallacious therefore to equate loss of potency in bio-assay with inactivation. In studying oestrogens in complex biological systems, a laboratory bio-assay response is an uncertain guide for the elucidation of any molecular change, or for distinguishing a degradative from a synthetic process.

Notwithstanding these difficulties some progress has been made. Studying the interconversion of steroids, Heard & Hoffman (1941) reported the isolation of oestrone from urine after administering oestradiol. Pearlman & Pincus (1943) isolated oestriol from male urine after the administration of oestrone. There is good evidence from in-vitro studies with human material for the ready interconversion of oestradiol and oestrone by a variety of tissues, including liver (Ryan & Engel, 1953). These authors noted the presence of other unidentified metabolites in their preparations, but were unable to isolate oestriol. Incubating oestrone in human blood mixtures, Levy (1954) found no conversion and was able to recover almost quantitative amounts of crystalline oestrone.

The recent isolation of previously unsuspected oestrogenic metabolites in human urine has been a major advance in allowing a tentative scheme for the metabolism of oestrone or oestradiol to be proposed. Marrian & Bauld (1955) reported the isolation from human pregnancy urine of a phenolic steroid differing from oestriol in the plane of the 16-hydroxyl group relative to the steroid nucleus. Evidence has been presented subsequently (Watson & Marrian, 1956), confirming that this steroid, 16-*epi*oestriol (XV), is naturally occurring and present also in non-pregnant female urine, but in very small amounts. In the same laboratory a further oestrogen metabolite, quantitatively more important than *epi*oestriol, has been isolated from human pregnancy urine and identified as 16α-hydroxyoestrone (XVI) (Marrian, Watson & Panattoni, 1957; Marrian, Loke, Watson & Panattoni, 1957). The most likely explanation of these and other known oestrogen metabolites is that oestrogenic hormone metabolism proceeds in the steps represented below:



A different urinary phenolic steroid, 16-keto-oestrone (XVII), has been reported by Serchi (1953), and Slaunwhite & Sandberg (1956) have isolated [4-^{14}C]16-keto-oestrone from luteal-phase urine after the administration of [4-^{14}C]oestradiol. If confirmed as a naturally occurring steroid, 16-keto-oestrone may perhaps prove to be an intermediate between oestrone and the epimeric 16-hydroxyoestrones.

The development by Brown (1955) of an accurate method for the individual estimation of oestrone, oestradiol and oestriol in low concentrations is an advance that will allow more precision and faster progress in metabolic studies. Using this method for the study of oestrogen excretion in liver disease, Cameron (1957) found that over-all increase of oestrone, oestradiol and oestriol was uncommon. This was surprising in view of the numerous reports in similar patients of increased oestrogen excretion as measured by bio-assay (Glass, Edmonson & Soll, 1940; Rupp, Cantarow, Rakoff & Paschkis, 1951; Pincus, Rakoff, Cohn & Tumen, 1951; Dohan, Richardson, Bluemle & György, 1952). These differences would be explained if the newly discovered oestrogens, *epi*-oestriol and 16α-hydroxyoestrone, have biological activity in the Allen-Doisy test and are also selectively increased in liver disease, for these newer steroids are not measured by the Brown method. Cameron (1957) noted a considerable increase in the proportion of oestriol relative to oestrone and oestradiol excreted in about half the patients with liver disease who were studied.

It can be concluded that, although there is a lot of experimental evidence connecting the liver with oestrogen metabolism in a general way, no unique function of the liver has yet been unequivocally demonstrated.

5. The Conjugating Function of the Liver

Steroid conjugates have been studied both because of their metabolic implications and for the problems they raise in the extraction of steroids from biological fluids. Steroids as a class are relatively insoluble in water and, in general, conjugation confers a marked increase in water solubility. Evidence has been presented for conjugation with sulphuric acid (Venning, Hoffman & Browne, 1942; Munson, Gallagher

& Koch, 1944) or as a glucuronide (Venning & Browne, 1936; Cohen & Marrian, 1936; Sutherland & Marrian, 1947). Although the primary hormones appear to be secreted in the free state, a number of conjugated derivatives have been found in blood and urine. In plasma, tetra-hydro derivatives of hydrocortisone occur mainly as glucuronides (Bongiovanni, 1954). Other plasma steroid conjugates have been suspected but not identified. In urine most of the steroid present is conjugated in the form of glucuronide and sulphate, although some appears to be in the free form. Small amounts of other conjugates have been reported.

Although it is generally held that the liver is important in the conjugation mechanism, experimental support for this belief is still weak. Szego (cited by Roberts & Szego, 1955b) has noted the formation of oestrogen glucuronides in in-vitro experiments with rat liver. However, Samuels *et al.* (1947) found no evidence to suggest that conjugation was a major factor in the inactivation of testosterone by rat-liver preparations. Studies of conjugation of steroids in patients with impaired liver function have not yielded consistent findings. Bongiovanni & Eisenmenger (1951) and Williams *et al.* (1951) reported results suggesting that in such patients there is an alteration in the conjugating process. West *et al.* (1951), however, found normal amounts of plasma conjugated 17-ketosteroid after testosterone administration. Many workers have reported an increase in urinary free oestrogen in patients with chronic liver disease, but Cameron (1957) found no increase above the normal negligible level of free oestrone, oestradiol, or oestriol, in such patients.

Most investigations of specific enzymes have dealt with their hydrolytic rather than their conjugative activity. β-Glucuronidases and sulphatases have been demonstrated in a wide variety of mammalian tissues (Mills & Paul, 1949; Kerr, Campbell & Levvy, 1949; Dodgson, Spencer & Wynn, 1956), but the direction of their activity under in-vivo conditions, whether hydrolytic or conjugative or both, is uncertain. The wide distribution of these enzymes makes it unlikely that the liver has any exclusive role in steroid conjugation. Moreover, the basic question whether the proximate activity of steroid hormones is associated with conjugation or hydrolysis of conjugates remains unanswered. The relationship between oestrogens and enzyme activity has been reviewed by Fishman (1951).

Evidence for the intervention of the liver in the binding of oestrogens to protein has been reviewed by Roberts & Szego (1955a). Protein binding of steroid (although differences of opinion exist as to the meaning of the term) is believed to occur. In rat liver an enzyme system appears to catalyse the binding of oestrogen to plasma albumin and liver protein, but the implication of these observations for human physiology is uncertain, as lipid transportation in man differs considerably from that in animals.

6. Clinical Aspects

The hypothesis that altered metabolism of steroids is a factor in the causation of some of the signs and symptoms in liver disease has been investigated by a number of workers.

There is no evidence to link the common changes in water and electrolyte balance with altered adrenocortical hormone metabolism. Although earlier workers reported normal or raised corticosteroid excretion in liver disease, Brown *et al.* (1954), using more specific methods, found 17-hydroxy-corticosteroid excretion reduced. The conclusion drawn by Brown and his colleagues was that this low excretion rate was the result of reduced adrenocortical secretion, a normal plasma corticosteroid level being maintained because of the slower rate of metabolism in the diseased liver. The high urinary aldosterone excretion in patients with ascites or oedema is found whether liver function is impaired or not (Luetscher, Deming & Johnson, 1952).

The relationship of salt retention and oedema in chronic liver disease to altered metabolism of oestrogens has been investigated by Preedy & Aitken (1956). They found retention of water, sodium, and chloride above normal after oestrogen administration in patients with cirrhosis and ascites, constrictive pericarditis, and, to a lesser extent, cardiac failure. This abnormal retention was not found in parallel experiments on patients with cirrhosis but without ascites, or with ascites due to peritoneal pathology. Preedy & Aitken postulated that, under conditions of impaired hepatic circulation, the metabolic inactivation of administered oestrogen was delayed, and by analogy they put forward the hypothesis that in liver disease altered inactivation of endogenous oestrogen is a factor in the production of ascites and oedema. Cameron (1957) found no correlation between the presence of ascites and increased excretion of free or conjugated oestrone, oestradiol, or oestriol.

Patients with chronic liver disease frequently experience alteration in their secondary sexual characteristics and sexual function, and urinary steroid studies have been carried out to link these clinical changes with changes in the metabolism of the sex hormones. The finding by Fraser, Forbes, Albright, Sulkowitch & Reifenstein (1941) that urinary neutral 17-ketosteroids are reduced in chronic liver disease has been repeatedly confirmed. 17-Ketosteroids from both testis and adrenal are reduced (Cameron, 1954). This is not due to impaired conversion of testosterone to 17-ketosteroids, as this conversion proceeds rapidly even in the presence of severe liver disease (Williams *et al.* 1951), but probably reflects a low production of androgens. Conflicting findings by different investigators of urinary oestrogen excretion in liver disease have already been discussed, and the widely held view that the impaired inactivation of oestrogenic hormones is responsible for the endocrine features of liver disease cannot be considered proved.

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THE CLINICAL PATTERN AND PROGNOSIS OF INFECTIVE HEPATITIS

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1 Clinical course
2 Convalescence and after-symptoms
3 Unusual forms of infective hepatitis
4 Treatment and speed of recovery
5 More serious sequelae and mortality
Reference

Infective hepatitis has long been wide spread throughout the world, tending always to be endemic and at times to become epidemic, frequently among troops in times of war. Interest in the disease drowsed along through the years, but was sharply awakened by the first of four massive outbreaks which occurred among troops massed round the Mediterranean shores in 1942 during the Second World War. This outbreak took place at a critical time in the campaign, shortly before the battle of El Alamein. Thousands of men were affected. The wastage of man-power was vast, not because of the severity of the disease but because of its long duration. In the six winter months of 1942–43, infective hepatitis cost the British troops in the Middle East over half a million man-days in hospital. Extensive studies were at once undertaken. It was confirmed that the morbid anatomical lesion was associated with diffuse damage of the parenchymal cells of the liver, without inflammation of the bile ducts. The cause was shown to be an infectious hepatotropic agent, presumably a virus. The clinical picture was defined as a clear-cut entity. Valuable epidemiological facts emerged. Since that time interest in the disease has continued.

1. Clinical Course

Infective hepatitis has a low mortality. The vast majority of patients recover completely, but the time they take to do so varies considerably. The clinical picture of the disease in its usual form is so well known that it would be superfluous to repeat it in detail. Suffice it to say that the onset of jaundice is frequently preceded by an initial period of illness, characterized by anorexia, malaise and sometimes fever, and enlargement of the liver. A few days later the urine is seen to be dark, or the patient jaundiced, or both. The stools may be white or grey. Soon the appetite returns, followed by a sense of reasonable well-being. The stools begin to regain their colour, the jaundice lessens and the urine becomes free from bile pigments. The duration of the disease tends to be longer as age advances. In children it may last only a few days. In young adults the majority of patients are free from jaundice and fit to return to work six or seven weeks from the onset of their symptoms. In older persons the disease may last much longer.

Diagnosis, especially during an epidemic, can be made almost at the onset by a precise knowledge of the initial symptoms. During the acute phase of the disease the empiric flocculation tests—such as the thymol turbidity test which depends on a rise in the γ-globulin fraction in the blood—may or may not be positive even when the attack is severe.

2. Convalescence and After-Symptoms

A small proportion of patients have a second attack of infective hepatitis shortly after the first, perhaps while they are still in bed or sometimes after an interval of about four weeks. The second attack has similar characters to the first attack.

Nearly all patients finally make a complete recovery. Convalescence is usually uninterrupted, but may be prolonged. In 1949, with the co-operation of the then Consulting Physician to the British Army, Brigadier J. Bennett, and of the War Office, and with the assistance of Dr J. S. Rivers and an expenses grant from the Medical Research Council, I began a large-scale follow-up of men who had suffered from infective hepatitis six or seven years previously in the Middle East epidemic of 1942–43. Taking, as far as possible, a random sample, I wrote to 1,293 men, and at that time 1,065 of them replied. The results have not yet been published, because the inquiry is being continued now, fourteen years after the event, to see if there is any evidence of late-developing cirrhosis. However, it was interesting to learn that 26% of the 1,065 men who replied had felt thoroughly fit after leaving hospital, 43% by the end of one month, 14% by the end of two months, 6% by the end of three months, 5% by the end of six months, 2% by the end of twelve months; and 4% had not felt perfectly well for more than one year afterwards.

These "post-hepatitis" symptoms tend to be vague and varied. The patients do not feel well. They may get unduly tired on exertion. There may be shortness of breath on effort. They may feel listless and have fits of despondency and be unable to make decisions. There may be times when they are unable to eat cooked fats and rich foods or to drink alcohol and they may even be revolted by the sight of them. Others experience aching in the muscles, or a feeling of coldness, with loss of appetite and nausea and a sense of great ill-being. This may be accompanied by discomfort or even pain in the right upper quadrant of the abdomen. They may say the symptoms are exactly the same as those they experienced at the beginning of their illness. During this time the liver may remain enlarged and the empiric flocculation tests may still be positive, but not always so. Frequently, the symptoms are made worse by hard exercise, fatigue, fatty foods and alcohol. Happily, all these symptoms nearly always disappear and perfect health is finally restored.

In a small minority of patients these symptoms persist for years. When the results of needle biopsy of the liver and biochemical tests are normal, these patients have sometimes been labelled as neurotics. At a time when talk of the possibility of the development of chronic hepatitis at a later date was rife, there is little doubt that fear occasionally aggravated the symptoms, but I do not for a moment believe that the symptoms are purely psychogenic in origin.

Some patients, who have symptoms for many years, develop what for want of a better term one calls the hepatobiliary syndrome. This syndrome, which is quite common, occurs of course in many people besides those who have

suffered from infective hepatitis. Certain groups may be recognized. Some are highly-strung individuals, with nervous dispositions. Others appear to have true biliary migraine. Sometimes, it affects people who have lived long in the tropics. Occasionally it is a sequel of amoebiasis. In many instances, however, no particular factor can be implicated.

The syndrome consists of attacks in which there is often nausea and lack of appetite, a general feeling of hebetude and malaise, a little fever and other symptoms, including headache and the passing of loose pale stools. Usually there is pain and tenderness in the abdomen, often localized, just below the tip of the right ninth costal cartilage. Attacks may last for a day or so and recur at irregular intervals over a long period of time. The symptoms may be precipitated by certain foods, particularly cooked fats, by worry and fatigue, by emotional upsets, but often by no known cause. The cholecystogram is essentially normal. It has been said that the syndrome is caused by biliary dyskinesia. Although the latter undoubtedly exists radiologically, there is little, if any, evidence that it is the cause of the hepato-biliary syndrome. It is true that the cholecystogram sometimes shows a rather slow-emptying gall-bladder in this condition, but usually the appearances are perfectly normal and it is seldom possible to correlate the functional changes seen in the cholecystogram with the symptoms. Patients suffering from the hepato-biliary syndrome respond quite well to medical treatment on a gall-bladder regime combined with reassurance, understanding and the treatment of symptoms. If the abdomen is opened, the gall-bladder is found to be normal and, if it is removed, symptoms persist as before. A note of caution should be sounded here. Infective hepatitis is not a prophylactic against the development of cholelithiasis which should always be considered when such symptoms are present.

In a very few patients, infective hepatitis is followed by bouts of slight jaundice which may persist for years without any evidence of serious liver damage or of deterioration.

In recent years interest has been taken in a " cholangiolytic cirrhosis " thought to be caused by inflammation of the fine cholangioles and smaller bile ducts, without any block of the larger bile passages. It has been said that this may sometimes follow infective hepatitis. If this is so, it must be rare. I have seen two doubtful examples, one patient who died after being jaundiced for ten years, and the other who is still alive; I am certain of neither.

It is important to stress that, although a patient may feel quite well after a recent attack of infective hepatitis, his liver may remain enlarged for months, and the biochemical tests remain positive for even longer. Lack of recognition of this fact leads to unnecessary alarm. Recovery is still the rule.

3. Unusual Forms of Infective Hepatitis

In some patients jaundice persists for a very long time. For example, I have seen a man remain deeply jaundiced for one year. He then made a complete recovery.

In the last few years, particularly among middle-aged women, transient ascites has sometimes been found to accompany what appears to be ordinary infective hepatitis. This feature, well known in Scandinavia, appears to be new to Britain. It was not remarked during the large epidemics of the Second World War. Its unfamiliarity has, on occasion, led to a mistaken laparotomy. In the cases I have seen convalescence has been prolonged but the ultimate prognosis good.

Infective Hepatitis without Jaundice

In an epidemic there is a large number of covert cases of infective hepatitis without jaundice. This has been proved by pathological tests and needle biopsy. When one comes to look for such cases, their clinical recognition is usually difficult or impossible. It should be said that patients who have infective hepatitis without jaundice or with very little jaundice may take as long or even longer to recover complete good health as those in whom the jaundice is great.

4. Treatment and Speed of Recovery

The effect of treatment on the speed of recovery and prognosis is difficult to assess. A common custom is to put adult patients to bed as soon as the disease has been diagnosed and to keep them there until their urine has been free from bile pigment for three successive days. They are then allowed to get up and potter about quietly for a week. They are regarded as infectious and nursed with full " barrier " precautions only until the temperature has become normal. If bile pigment reappears in the urine they are put back to bed. After the patients have been up for one week with no bile pigment in the urine, they are sent for a physically restful holiday for at least a fortnight.

In the early stages of the illness feeding may be a big problem. The patients may have such intense dislike for food that one has to give them whatever they can be persuaded to eat. When appetite returns, however, they are given a good attractive generous mixed diet containing plenty of protein and carbohydrate in the form of sugar. Fats are not restricted, unless the patients find them distasteful, which they often do when the fats are cooked. Added methionine and choline are considered unnecessary and not given.

This generous feeding may have no scientific basis in well-nourished human beings, except that the patients may have lost a lot of weight, but it does seem to speed up recovery.

No specific therapy for infective hepatitis has yet been found. Antibiotics appear to have no value in treatment of the disease. Medicinally, saline purgatives are given for the bowels if required. Chloral hydrate or paraldehyde are used when hypnotics are needed. Morphine and barbiturates are not prescribed. Alcohol is strictly forbidden. There appear to be no contra-indications to using sulphonamides or antibiotics for the treatment of intermittent infections if they arise.

With impending coma the regime is different. The importance of restricting protein and the possible value of antibiotics are discussed elsewhere in this number of the Bulletin.

One regards the after-treatment of infective hepatitis as being extremely important. Two things are forbidden absolutely to the patients until such time as their livers have become impalpable, their blood biochemically normal, and they themselves feel one hundred per cent fit. These are alcohol and hard exercise. Failure to observe these restrictions may delay recovery.

Alcoholics are no more likely to acquire infective hepatitis than teetotallers and, when they do, the disease does not run a more severe course. But it is generally agreed that, during the acute stage and during convalescence, alcohol is most harmful. Tolerance is less. Moreover, an unwise drink may be followed the next day by the reappearance of bile in the urine. Some observers consider that alcohol plays a part in bringing about a second attack.

It should be recalled that the liver may remain palpable and the empiric flocculation tests remain positive for weeks or months after the patients feel perfectly well. As a general rule, one forbids alcohol and hard exercise to adults for at least six months in any event.

This regime, with minor variations, is widely followed. Recently, however, the importance of rest has been questioned. Chalmers, Eckhardt, Reynolds, Cigarroa, Deane, Reifenstein, Smith & Davidson (1955) describe investigations carried out in a United States Army centre in Japan set aside for the treatment of soldiers suffering from infective hepatitis contracted in Korea and south-western Japan; 460 patients were studied. Chalmers and his colleagues came to the following conclusions. Firstly, that recovery took place faster when patients were made to eat a full diet high in calories, proteins and vitamins than when they ate a light diet. Secondly, that patients fed on a high-protein diet recovered more rapidly than those fed on a moderate-protein diet. Thirdly, that there was no difference in the duration of the acute stage of the illness between those patients who were confined strictly to bed and those who were allowed up in the ward to do as they pleased. The latter patients were followed up for one year and during that time no significant difference between the two groups was noticed with regard to residual symptoms. Fourthly, that patients who were sent, after the acute stage of the illness was over, to a reconditioning centre where they underwent considerable physical exertion, did not come to any positive ultimate harm. They did find, however, that these patients at the time developed far more symptoms and minor abnormalities such as right upper abdominal pain, enlarged and tender livers, increase of serum bilirubin level, and pathological changes in the bromsulphalein test, than those patients who had a leisurely convalescence.

The weakness of these investigations lies in the fact that the various treatments were not begun until an average of ten days from the appearance of dark urine. This delay is inevitable, and it was the great stumbling block in attempts to study the effects of treatment on the speed of recovery and on the prognosis during the epidemics of the Second World War. Quite apart from the impossibility of forecasting the ultimate severity of a particular case at the beginning, it is difficult to assess the value of any special therapy because, by the time patients are admitted to hospital, the worst of their symptoms are so often over. For example, in one centre in the Lebanon, in 1942, where 78 cases of infective hepatitis were admitted as early as possible after diagnosis, the average (mode) days on which events occurred from the onset of symptoms were as follows: appearance of dark urine or jaundice, the 4th day; admission to hospital, the 7th day; return of appetite, the 8th day; return of sense of well-being, the 12th day; urine first free from bile, the 17th day; getting up from bed, the 20th day; and leaving hospital for convalescence on the 34th day. Variation in the length of the illness is shown in this series because, whereas the average (mode) day of leaving hospital was the 34th, the days of departure for all these patients ranged from the 11th to the 101st day. For these reasons one concludes that more precise methods of assessment are needed than evidence of clinical improvement alone. Personally, I should be very loath at the present time to jettison the admittedly empiric but well-tried programme of rest, diet and abstinence already outlined.

5. More Serious Sequelae and Mortality

If one regards acute and subacute necrosis of the liver and possibly late-developing cirrhosis as extensions of infective hepatitis, complications of the disease are negligible and more or less non-existent. The *mortality* of infective hepatitis is generally extremely low. It is difficult to estimate when the disease is only endemic, but in the big Middle East epidemics it was about 0.2%. Death results from hepatic necrosis.

Acute necrosis of the liver is almost invariably fatal, death occurring within the first few days or weeks from the onset of hepatitis. The symptoms are discussed elsewhere in this volume. Nothing in the character of the attack of hepatitis at its onset gives warning of the advent of acute necrosis, and symptoms last only a few days before death.

It is hoped that new methods of treatment may sometimes modify the outcome.

Subacute necrosis of the liver, associated with jaundice, is seen not infrequently in practice. Occasionally, it follows an attack of infective hepatitis, but whether there are other causes, and what they are, we do not know. The condition is seen mostly in adults. It is characterized by attacks of jaundice, and the initial symptoms and signs are indistinguishable from those of infective hepatitis. Jaundice may wax and wane for weeks, or months, or even years. It may come in attacks at intervals which vary from weeks to many years, and at any time may end in hepatic failure and death, or may end in recovery. Episodes of coma in this condition are sometimes dramatically controlled by modern treatment, with protein restriction. If the disease lasts for many years or if many years elapse between attacks, the final morbid picture in the liver is one which most pathologists find indistinguishable from that of portal cirrhosis.

Portal cirrhosis (*Laennec's*) appears to be the final pathological result of many different processes. The clinical picture of portal cirrhosis (Laennec's) should be separated from that of long-standing subacute necrosis of the liver. The final pathological changes in the liver in the two conditions may be indistinguishable from each other, but the clinical pictures are very different. In subacute necrosis of the liver there is a story of jaundice, often in a series of attacks which, at least in their earlier phases, show clinical and pathological evidence of single, spaced, violent assaults on the liver cells. In portal cirrhosis (Laennec's) the early stages are quiet and long. The complete clinical picture occupies only a relatively short space of time in the later stages of the disease. It is thought that this latter condition may occasionally be a late sequel of infective hepatitis. That this often happens is difficult to believe, but it is hoped that the follow-up referred to above, now fourteen years from the date of the attacks of infective hepatitis, may help to elucidate the point. Even allowing for the fact that an attack of infective hepatitis without jaundice, which a patient may have known nothing about, may have consequences as serious as—or more serious than—a florid attack, one would surely expect a past history of jaundice in patients suffering from cirrhosis more often than in the general population of the same age-groups. That is not my own experience.

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HAEMOCHROMATOSIS

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1 Clinical features
2 Iron metabolism in general
3 Iron metabolism in haemochromatosis
4 Prognosis
5 Treatment
References

The word haemochromatosis was coined by von Recklinghausen (1889), though the first undoubted case had been reported previously by Trousseau (1865). It is a rare disease, and Sheldon (1935) was able to collect from the world literature records of only 311 patients in whom this diagnosis could be accepted as probably correct. It is now clear, however, that the disease is commoner than Sheldon's figures suggest and that the 222 cases collected from the literature between 1935 and 1952 (Houston, 1953) gave far from a complete list of the patients reported during that period. There are two main reasons for the apparent increase in the incidence of the disease. Interest in haemochromatosis, stimulated by the publication of Sheldon's monograph in 1935, was enhanced a few years later by the rapid advances being made in the understanding of the metabolism of iron, and increased awareness about a disease invariably brings to light previously unsuspected cases; and, secondly, there are now available procedures which enable the diagnosis to be established long before it would be certain on purely clinical grounds. The true incidence of the disease is hard to estimate. Bell (1955) found 58 cases among 932 patients with clinical portal cirrhosis coming to autopsy, and lesser degrees of haemosiderosis in many of the other patients; he concluded that haemochromatosis is not a sharply defined entity, but blends gradually with ordinary portal cirrhosis. This interesting suggestion would certainly not be generally accepted at the present time, and the remarkably high incidence of haemosiderosis found in Bell's cirrhotic patients has not been reported by other workers.

1. Clinical Features

The clinical picture of haemochromatosis is well known, its most prominent features being pigmentation of the skin, cirrhosis of the liver and hypogonadism. Diabetes mellitus occurs in only about 70% of the cases, so that the old term " bronzed diabetes " can no longer be regarded as an appropriate label and has been generally discarded. Impotence and lethargy are common presenting symptoms; other patients seek medical advice in the first place for the associated diabetes, while not infrequently the loss of body-hair, dusky skin and enlarged liver are noted at routine clinical examination before any significant symptoms have appeared. The skin is usually thin, dry and atrophic. The diffuse greyish-brown pigmentation, which may be seen over the whole body but is particularly striking on the face, neck and forearms, is due mainly to increased melanin in the basal layer of the epidermis and only partly to deposition of haemosiderin. Indeed there may be no excess of iron to be seen histologically in some areas of pigmented skin, and for this reason skin biopsy is not a satisfactory diagnostic procedure. On the other hand, gross impregnation with iron-containing pigment is the cardinal histological feature of many of the internal organs, notably the liver, the pancreas and the heart; thus the total body-iron, which in normal subjects is about 3–5 g., may amount in patients with haemochromatosis to 50 g. or more.

2. Iron Metabolism in General

Present views on iron metabolism and its disorders stem from the work of McCance & Widdowson (1937, 1938) who pointed out that, contrary to the previous belief, only negligible quantities of iron are excreted by the colon. They originally made this deduction from the well-known fact that when a large amount of iron is liberated by abnormal haemolysis, or accumulates because of a hold-up in erythropoiesis as in pernicious anaemia, the excess is not excreted but is deposited in the tissues; and they subsequently demonstrated that when iron is given by mouth to experimental animals it can all be recovered from the faeces, but when it is given intravenously it can all be found in the organs. As very little iron is excreted in the urine, it soon became clear that there must be a mechanism to prevent excessive absorption of iron from the gut; the mucosal block theory, put forward by Hahn, Bale, Ross, Balfour & Whipple (1943) and largely confirmed by Granick (1946), still remains the best explanation of the known facts on this subject. This theory postulates that iron absorbed from the lumen of the small intestine, particularly the duodenum, unites reversibly with the protein apoferritin to form ferritin; when a certain concentration of ferritin is reached in the mucosal cells no more iron can be taken up until some of the mucosal ferritin has yielded up its iron to the blood plasma. Iron lost from the body, in the urine, in bile, and in desquamated cells, does not exceed 1.0 mg. daily, and normally the mucosal block mechanism limits iron absorption to the amount necessary to cover this loss. In this way the available stores of iron in the body are kept fairly constant, at a level usually somewhat below 2 g.

There is clinical and experimental evidence, however, that under certain conditions the mucosal block mechanism fails to work satisfactorily so that an excess of iron is absorbed and added to the body's stores. The first of these conditions is the ingestion of large quantities of iron. Brock & Hunter (1937) showed that the administration of large doses of iron by mouth was followed by excessive iron absorption; a diet containing up to 200 mg. daily is thought to be an important factor in the production of gross haemosiderosis in the Bantu (Walker & Arvidsson, 1950; Gillman & Gillman, 1947); and children poisoned with ferrous sulphate, which they have eaten in mistake for sweets, have been found to have serum iron levels as high as 1 mg./100 ml. It has been shown that rats absorb more iron from a diet which is low in phosphorus and less from a diet rich in phosphorus (Kinney, Hegsted & Finch, 1949; Hegsted, Finch & Kinney, 1949), but this factor is of doubtful importance in man, since the Bantu natives have a high iron absorption in spite of the high phosphorus content of their diet (Walker, 1951). It has also been shown

experimentally that vitamin A deficiency, or pancreatectomy, or ligature of the pancreatic duct, leads to hepatic siderosis in cats, presumably as a result of excessive iron absorption (Taylor, Stiven & Reid, 1935), but whether these findings have any relevance to human siderosis is unknown. Finally, anaemia may be accompanied by increased iron absorption (Dubach, Callendar & Moore, 1948; Houston, 1951).

3. Iron Metabolism in Haemochromatosis

In haemochromatosis the stores of iron in the body are grossly overloaded, and it is clear that there has been excessive iron absorption for many years. The increased absorption can indeed be demonstrated by using a radioactive iron technique; in patients with haemochromatosis a smaller proportion of the ingested dose of ^{59}Fe can be recovered from the faeces than in normal subjects. On the other hand, the patient with haemochromatosis synthesizes a much smaller proportion of the absorbed ^{59}Fe into haemoglobin, presumably because it is diluted in a much larger pool of available iron. There can be no doubt therefore about the fact of excessive iron absorption in this disease, but its cause remains an unsolved problem. Some of the factors already discussed that are known to increase iron uptake may play some part, but only in a small group of atypical cases: protein deficiency and excessive dietary iron in the Bantu (Gillman & Gillman, 1947; Walker & Arvidsson, 1950); and chronic anaemia, occasionally even in patients who have received no blood transfusion (Houston, 1951), but particularly when repeated transfusions have added to the accumulating deposits of iron in the tissues (Kark, 1937; Schwartz & Blumenthal, 1948). In the great majority of patients with haemochromatosis, however, there is no evidence that the diet has contained too much iron or too little of any other essential constituent and there is no history of anaemia or blood transfusions: the cause of the increased iron absorption is unknown.

The only available evidence with a possible bearing on this problem suggests that constitutional factors are important and that the excessive iron uptake may be the result of an inborn error of metabolism. Thus, sparsity of body-hair characteristically dates from puberty in these patients, usually preceding the other clinical features of the disease by many years, and improved diagnosis is revealing that multiple cases in a family are fairly common. Althausen, Doig, Weiden, Motteram, Turner & Moore (1951) found two pairs of siblings with the disease among their 23 cases investigated in Melbourne; Houston & Zilkha (1955) described a woman and her twin sons, in all of whom this diagnosis was established, and I have subsequently seen the disease in its fully developed form in two brothers.

Haemochromatosis is very rare in women, in whom it tends to appear at a later age than in men. Of 512 patients reported in the literature only 33 were women and, whereas the men were fairly evenly distributed throughout the adult years of life, only three of the women were under the age of 45 (Houston, 1953). During the 30 years of reproductive life the average loss of iron through menstruation and pregnancy is probably about 10–15 g.; and, assuming a similar incidence in both sexes of the underlying metabolic defect of haemochromatosis, the elimination by women of this quantity of iron may be sufficient to delay or prevent the clinical expression of the disease. In this connexion it is of interest that, when haemochromatosis does occur in young women, there is frequently a long history of scanty periods or amenorrhoea (e.g. Lyon, 1936).

King & Downie (1948) stressed the value of liver biopsy in the diagnosis of haemochromatosis, and this procedure is now generally accepted as a satisfactory way of establishing the diagnosis. It is not entirely free from risk, however, and cannot be performed in out-patient or domiciliary practice, and serum iron estimations provide an equally reliable but simpler and safer way of confirming or refuting the diagnosis (Houston & Thompson, 1952, 1953). Iron does not exist in the free state in the serum, but is transported in combination with a specific fraction of the β-globulin. The total iron-binding capacity (TIBC) represents the total amount of iron that the iron-binding globulin can take up. Normally the TIBC is about 300–350 μg./100 ml., so that the normal serum iron level of about 100 μg./100 ml. (range 50–150 μg.) represents approximately one-third saturation of the serum iron binding capacity. In haemochromatosis the serum iron level is above the normal range, the TIBC is somewhat lower than normal and there is complete or almost complete saturation of the iron-binding capacity; the only other condition in which this biochemical picture is seen is transfusion haemosiderosis. On the other hand, high serum iron levels may be due to increased catabolism of haemoglobin in haemolytic anaemia, to reduction in haemoglobin synthesis by factors other than lack of iron, for example pernicious anaemia in relapse and aplastic anaemia, or to liver disease such as infective hepatitis or—less often—cirrhosis, in which the liver cells either fail to store iron or actually liberate their iron into the serum by disruption; but in none of these conditions is there the high degree of saturation of the iron-binding capacity which characterizes haemochromatosis.

4. Prognosis

The prognosis of the disease is extremely hard to estimate and many years must elapse before adequate data become available on the course of the disease as modified by current methods of treatment. Sheldon (1935) found that the average length of survival from the time medical advice was first sought until death was 18½ months, this estimate being based upon a study of 89 cases recorded at that time. The very short duration of the disease in this series is due to the fact that most of the patients lived before the discovery of insulin and died of diabetic coma; with adequate control of the diabetes most patients live for at least a number of years. The commonest causes of death in this disease nowadays are hepatic failure and cardiac failure. A small proportion of the patients develop primary carcinoma of the liver: Warren & Drake (1951) found the incidence to be 18.9% in haemochromatosis compared with 4.4% in cirrhosis; Berk & Lieber (1941) put the incidence in haemochromatosis at 7%.

Three of my own patients have died within the past few years: one from progressive hepatic failure with terminal massive bleeding from oesophageal varices; one from congestive cardiac failure; and the third a few hours after the abrupt onset of severe cardiac pain and profound shock. Myocardial infarction was the clinical diagnosis, but autopsy revealed only a terminal sero-fibrinous pericarditis and heavy infiltration of the myocardium with iron. McClatchie, Taylor & Henry (1950) reported a man with haemochromatosis who died within 24 hours of developing acute abdominal

pain and severe irreversible shock. At post-mortem examination no cause for the terminal episode was found and the authors suggest that the severe shock may have been due to release into the blood stream of ferritin, which Mazur & Shore (1948) showed to be identical with their vasodilator material (VDM) of hepatic origin. Sudden cardiovascular collapse, without pathological evidence of coronary occlusion or any other cause, also occurred in three out of the 33 cases of haemochromatosis and cardiac involvement which were collected by Bothwell & Alper (1951), though by far the commonest evidence of heart disease in this group was congestive cardiac failure.

5. Treatment

It has been clearly established that some at least of the excessive iron stores in haemochromatosis are available for haemoglobin synthesis and can be removed by repeated bleeding, and regular venesection has become part of the standard treatment for patients who are in sufficiently good general health at the time the diagnosis is made. It appears probable, though it cannot yet be regarded as proved, that removal of this available iron may slow down or stop the deterioration in function of the liver and the heart, the two organs whose involvement is at present responsible for most of the mortality and much of the morbidity of the disease. Very large quantities of blood can be withdrawn from these patients without causing anaemia or other ill effect: the first of my patients treated in this way (Houston, 1953) had 82 pints of blood removed in two-and-a-half years before his blood count showed early signs of iron deficiency. When this stage is reached presumably all the iron which is physiologically available has been removed. Bleeding should then be continued at a reduced rate to keep the haemoglobin just below the normal level, with the object of preventing reaccumulation of iron in the tissues.

In addition to regular blood-letting, about two-thirds of all patients with haemochromatosis have diabetes mellitus and require treatment with diet and insulin; and many also derive great benefit from implants of testosterone propionate, 600 mg. every 3–6 months, which not only relieve impotence but help in restoring general strength and well-being.

In conclusion it may be said that, while the past twenty years have seen great advances in the understanding of this disease and in its treatment, so that the views current at the time of Sheldon's monograph are now remarkably out-dated, nevertheless its cause remains unknown, its treatment is crude in method and unpredictable in effect, and we need once more the impetus of a new approach to this problem.

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HEPATOLENTICULAR DEGENERATION (WILSON'S DISEASE)

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1 The clinical syndrome
2 The biochemical lesion
3 Diagnosis
4 Treatment
References

Although hepatolenticular degeneration is primarily a metabolic disease, the rather dramatic nature of the nervous symptoms have, until recent years, resulted in this condition's remaining largely in the province of the neurologist. It is only in the last decade, with the increasing introduction of biochemical techniques into clinical medicine, that fundamental advances have been made in knowledge of its pathogenesis and treatment.

1. The Clinical Syndrome

While the tremor and mental changes which may develop in certain cases of chronic liver disease may mimic those found in the earlier stages of hepatolenticular degeneration, the resemblance is seldom so close as to cause difficulty in diagnosis. In his original account of six cases of progressive lenticular degeneration, Wilson (1912) described the association of hepatic cirrhosis with symmetrical degeneration of the putamen, globus pallidus and caudate nucleus. His patients all showed tremor, muscular rigidity, dysarthria, dysphagia and emotional lability; he also noted that the disease had a high familial incidence. It is now known that, in addition to the basal ganglia lesions, the cerebral cortex is also affected by the disease process, and an important additional clinical finding is the characteristic brownish Kayser-Fleischer ring at the corneal margin. Hepatolenticular degeneration is a disease of children or young adults; it has no specific sex incidence, but often more than one sibling in a family is affected and Bearn (1953) found that, in as many as one-third of the cases he investigated, the parents were cousins. He believed that the disease was inherited as an autosomal recessive. In infants and young children the presenting symptoms may be those of hepatic disease, and the diagnosis of hepatolenticular degeneration should always be considered in otherwise unexplained juvenile cirrhosis. In adolescents tremor is commonly the initial symptom, often diagnostic in character for, while at rest it is of a parkinsonian type, on purposive movement a wild ataxia develops. In addition there may be choreiform movements. Often these unfortunate patients are labelled as hysterics because of the exaggerated character of the tremor. As the disease progresses, muscular rigidity and contractures come to dominate the picture, and emotional disturbances or some mental deterioration may occur. Concurrently with these changes dysarthria develops, there is inability to close the mouth so that the face has a fixed grin, and drooling becomes a constant and distressing symptom. In the early stages, and often till quite late in the disease, evidence of liver damage is not readily found and even liver function tests may be negative or only weakly positive, though it is possible that a biopsy would almost always show histological changes. The duration of the illness in this age-group is usually from six months to two years from the onset of symptoms, but may occasionally be rather longer. In cases presenting in the third decade—and the disease seldom manifests itself later—besides the typical tremor, evidence of chronic liver disease is apt to be well marked and the patients may die either from progression of the basal ganglia lesion, from liver failure, or following haemorrhage from oesophageal varices.

The pathology of the cerebral lesions has been described by Greenfield, Poynton & Walshe (1924). There is no characteristic finding in the liver (see Plate IV, fig. A), fibrosis may be present as either diffuse hepatic fibrosis (cf. case number 2, fig. 56, Wilson, 1912) or post-necrotic scarring (Wilson, case number 3, fig. 60), and both types of lesion may be present in the same liver.

2. The Biochemical Lesion

Within a year of Wilson's original publication, Rumpel (1913) offered the first clue to the nature of the biochemical lesion when he reported finding a significant increase in the copper content of the liver of a patient dying of this disease. A similar observation was made for the brain as well as the liver, in single cases, by Haurowitz (1930), Luthy (1931) and Glazebrook (1945), and in a series of three cases by Cumings (1948). At first it was thought that the copper concentration in the plasma was also high (Glazebrook, 1945; Cumings, 1951; Matthews, Milne & Bell, 1952), but it is now known that it is almost invariably low (Bearn, 1953; Cartwright, Hodges, Gubler, Mahoney, Daum, Wintrobe & Bean, 1954). Such an abnormality is also found in the nephrotic syndrome where, however, it is known that copper is lost in direct proportion to the proteinuria (Cartwright *et al.* 1954; Markowitz, Gubler, Mahoney, Cartwright & Wintrobe, 1955). Another constant finding in Wilson's disease is the very large amount of copper excreted in the urine; this may exceed the normal by tenfold or even twentyfold (Bearn & Kunkel, 1952; Cartwright *et al.* 1954) (see also Table I). Besides the cupruria there is an aminoaciduria and often a glycosuria of renal origin (Uzman & Denny-Brown, 1948; Cooper, Eckhardt, Faloon & Davidson, 1950). A rough correlation between the excretion of amino acids and of copper was reported by Cumings (1951), and Matthews *et al.* (1952) reported that they were able to increase the urinary copper in two patients with Wilson's disease by giving large doses of glycine or alanine. They believed that the urine copper was derived from ionized plasma copper filtered at the glomerulus and, assuming that all copper so filtered was excreted, they calculated that 1% of the plasma copper was present in the ionized state.

The year 1952 saw the publication of two papers which have given rise to the current views on the pathogenesis of Wilson's disease. Scheinberg & Gitlin reported that ceruloplasmin, the blue copper-binding α-globulin first described by

TABLE I. COPPER METABOLISM IN WILSON'S DISEASE

Estimation		Normal		Wilson's disease		Reference
		Mean	Range	Mean	Range	
Urine Cu (μg./24 hr.)		9 48	0–26 ±16.3	302 703	119–611 ±308	Cartwright *et al.* (1954) Bearn & Kunkel (1954)
Plasma Cu (μg./100 ml.)	Total plasma Cu	116 108 108	68–161 92–123 ±9.8	50 59 60	33–65 43–100 ±15.4	Cartwright *et al.* (1954) Markowitz *et al.* (1955) Bearn & Kunkel (1954)
	Direct plasma Cu	8 5	0–20 0–17	26 32	12–41 11–50	Cartwright *et al.* (1954) Markowitz *et al.* (1955)
Plasma ceruloplasmin (mg./100 ml.)		30* 34	— 27–38	20 9	— 2–19	Scheinberg & Gitlin (1952) Markowitz *et al.* (1955)
Cerebrospinal fluid Cu (μg./100 ml.)		6	3–9	18	10–28	Cartwright *et al.* (1954)

* Immunochemical method

Holmberg & Laurell in 1947, was deficient or absent in patients with hepatolenticular degeneration. This important observation has been extended and elaborated to cover most of the observed facts by two principal groups of workers, Bearn and Kunkel at the Rockefeller Institute in New York, and Cartwright and his associates in Salt Lake City. The second theory was first proposed by Uzman & Hood (1952) and has been elaborated in subsequent papers by Uzman and his associates (Uzman, 1953; Uzman, Iber, Chalmers & Knowlton, 1956). Originally Uzman studied a case of hepatolenticular degeneration with five asymptomatic siblings; all had aminoaciduria and peptideuria and this, he believed, was the primary metabolic defect. Later Uzman (1953) proposed the following theory. In patients with Wilson's disease, abnormal tissue proteolytic enzymes are present which lead to the intracellular accumulation of oligopeptides with dicarboxylic residues; by oligopeptides, it is presumed that Uzman meant peptides of low molecular weight. These form complexes with copper, for which they have a strong affinity, and the peptide–copper complexes so formed, principally in the cells of the liver and brain, overflow into the plasma and are excreted at the glomerulus. As a result of this peptideuria there is competition with amino acids for reabsorption by the renal tubules but, as the peptide–copper complexes are preferentially reabsorbed, the amino acids are excreted, giving rise to a raised renal clearance for amino acids. However, according to this theory, some peptides are also excreted resulting in a characteristic peptideuria and cupruria. The first detectable sign of the disease, then, is aminoaciduria which in due course leads to hepatic cirrhosis. In their most recent publication, Uzman and his associates (Uzman *et al.* 1956) claim to have isolated from the liver of a patient with Wilson's disease a fraction which, when separated by electrophoresis, stained blue with ninhydrin, showed a very high affinity for ionized copper and was not found in normal liver or in the liver of patients with other types of cirrhosis. The presence of this compound, they allege, is the genetically determined biochemical abnormality in this disease, leading to the formation of proteins with a very high affinity for copper. The excess absorption of copper from the gut in Wilson's disease could then be explained by postulating that this compound is also to be found in the intestinal mucosa. The presence of this tight copper–protein complex in the cells would then make the presence of ceruloplasmin unnecessary, or alternatively, block its formation, giving rise to the characteristic finding of a low plasma level of this protein. This theory is unsatisfactory since it is not fully in keeping with the observed facts. First, if all the copper excreted is peptide-bound, then 400 μg. of copper (an average daily figure for a patient with Wilson's disease) would be bound by 600 μg. of dicarboxylic residues (if each dicarboxylic grouping mol. wt. 90 binds one atom of copper at. wt. 63). Now if such an oligopeptide contains, say, 20 amino-acid radicles, then 400 μg. of copper would be bound to approximately 18 mg. of peptides per 24 hours. Stein, Bearn & Moore (1954) state that normal urine can liberate about 1.5 g. of amino acids from conjugated linkages on hydrolysis. In other words, the amount of peptide–copper complexes postulated by Uzman could not possibly account for a renal aminoaciduria by competition at the tubular level. In any case it is improbable that copper–peptide or copper–amino-acid complexes would compete with the free compounds for reabsorption by the renal tubule. Second, it is now established that aminoaciduria is not the first detectable abnormality in Wilson's disease, and fully developed cases have been reported with a normal excretion of both amino acids and peptides (Stein *et al.* 1954; Cartwright *et al.* 1954); moreover, no other workers have reported finding asymptomatic siblings with abnormal urinary amino acids. Third, the absence of ceruloplasmin is hardly explained by the theory that there is no plasma copper to be bound since, in the majority of cases of Wilson's disease, there is a plasma copper concentration of 40–60 μg./100 ml. and one-half to two-thirds of this is "direct reacting", that is, not bound to ceruloplasmin. In other words, the concentration of unbound copper is three times higher in patients with Wilson's disease than in normal

persons (Cartwright *et al.* 1954). Fourth, the slow disappearance of intravenously injected radioactive copper (^{64}Cu) from the plasma of patients with Wilson's disease hardly seems compatible with the theory that the tissue proteins have an abnormally high avidity for copper, as these would be expected to take up copper more rapidly, not more slowly, than normal. Finally, the loss of excess amino acids in the urine does not explain the hepatic cirrhosis, since not only does hepatic cirrhosis occur in those cases in which there is no aminoaciduria but also the other low threshold aminoacidurias are not constantly associated with liver damage. Hepatic cirrhosis is not part of the syndrome of classical cystinuria or the newly described Hartnup disease (Baron, Dent, Harris, Hart & Jepson, 1956), nor is it more than a rare accompaniment of the Fanconi syndrome. In his records of 55 cases of this latter disease Professor C. E. Dent (personal communication) has found only six examples with hepatic cirrhosis, and five of these belonged to a clearly defined subgroup, not yet fully described. There is no evidence from these figures to support the theory that a renal aminoaciduria can, by itself, lead to hepatic cirrhosis. These then are the criticisms which the theory of Uzman and his associates has failed to meet satisfactorily.

The alternative theory, based on the work of Scheinberg & Gitlin (1952), has been ably stated by both the New York and the Salt Lake City groups of workers. It may be summarized as follows. The primary biochemical defect in hepatolenticular degeneration is a failure to synthesize the α-globulin ceruloplasmin. This is a blue serum protein, mol. wt. 151,000, each molecule of which binds 8 atoms of copper. It is present in the plasma of normal individuals in a concentration of 20–30 mg./100 ml., but in patients with Wilson's disease the concentration is very greatly reduced and the protein may be absent altogether. Associated with this, though not necessarily secondary to it, is an excess absorption of copper from the gut. Owing to the deficiency of ceruloplasmin, copper is not tightly protein-bound and remains as free or direct reacting copper, loosely complexed to albumin. This allows it to be deposited in the tissues, where it accumulates. The organs principally affected are the liver, leading to cirrhosis; the brain, leading to cellular death and gliosis; and the kidney, leading to aminoaciduria and glycosuria. With the passage of time more and more copper accumulates in these organs where it is chelated by amino acids and peptides and overflows into the plasma; there it is present as direct reacting copper and is finally excreted in increased amounts in the urine. Studies with ^{64}Cu by both these groups of workers and by Earl, Moulton & Selverstone (1954) have all supported this hypothesis. Radioactive copper, when given either by mouth or vein to normal subjects, rapidly reaches a peak concentration in the blood, attached to serum albumin. It then disappears, only to return some 12 hours later bound to the ceruloplasmin. In patients with Wilson's disease this biphasic curve is not seen; having reached a peak, the copper concentration then falls off very slowly. As compared with normal subjects, much of this copper is excreted in the urine, very little in the stools, and a high percentage of the total dose is retained in the body. It has been estimated that, when on a normal diet, patients with Wilson's disease may be in positive copper balance by as much as 2 mg. daily. The only serious criticism of this theory of the pathogenesis of Wilson's disease is that it fails to account for the excess absorption of copper from the gut. If it is accepted, and on the present evidence it must be, it places Wilson's disease alongside haemophilia and agammaglobulinaemia as a disease characterized by failure of synthesis of a single serum protein.

As an extension of this theory it has recently been suggested that, in patients with Wilson's disease, copper accumulates in those organs with most Krebs-cycle activity (Walshe, 1956b) and that this is due to the avidity of one of the enzymes of this system, possibly with a sulphydryl group, for the direct reacting copper which is present in excess in the plasma of these patients. Not only would this account for the deposition of the copper in the brain, liver and kidneys, but it would also explain the distribution of copper in the brain; those areas with the greatest neuronal activity, the cerebral cortex and basal ganglia, being those principally affected. It is perhaps of some interest that the chicken, an animal with little or no ceruloplasmin, is particularly susceptible to excess copper in the diet, which leads to a marked accumulation of copper in the liver (Goldberg, Williams, Jones, Yanagita, Cartwright & Wintrobe, 1956).

3. Diagnosis

The main differential diagnoses are between familial and hysterical tremor, juvenile parkinsonism, and the tremor often found in association with chronic liver damage. The character of the tremor should be the clue to the diagnosis of the disease; in hepatolenticular degeneration it is parkinsonian at rest, greatly exaggerated and of a cerebellar type on purposive movement. The finding of Kayser-Fleischer rings at the corneal margin is diagnostic. Liver damage may not be marked and the routine liver function tests are not of diagnostic value, although the cephalin flocculation test is often positive (Franklin & Bauman, 1953). Of far more importance are studies on copper metabolism. In the absence of gross proteinuria, a 24-hour copper excretion of over 200 μg. is found only in Wilson's disease and certain types of chronic liver disease, particularly biliary obstruction and biliary cirrhosis. However, in patients with primary liver injury, unlike those with Wilson's disease, the plasma copper concentration is raised in proportion to the raised urinary excretion of copper. The urinary copper can be readily estimated by the method of Earl (1954). A low or absent ceruloplasmin plasma is a finding which will confirm the diagnosis, and this too can be quickly estimated by a rough method recently described by Ravlin (1956). The demonstration of a high urinary copper excretion in the absence of proteinuria and in association with a low or low-normal plasma copper level, will also confirm the diagnosis. It is not necessary to show, in addition, that there is an aminoaciduria of renal origin.

4. Treatment

If the symptoms of hepatolenticular degeneration are caused by an accumulation of copper in the brain and liver, it would be natural to expect that removal of this excess copper would at least prevent the progress of the disease or, if started early enough, ameliorate the symptoms. For this to happen, however, therapy must be started before irreversible damage has been done to the cerebral cortex and basal ganglia, for once neurones are destroyed they cannot be replaced. It was first shown by McCance & Widdowson

HEPATOLENTICULAR DEGENERATION

(WILSON'S DISEASE)

J. M. Walshe

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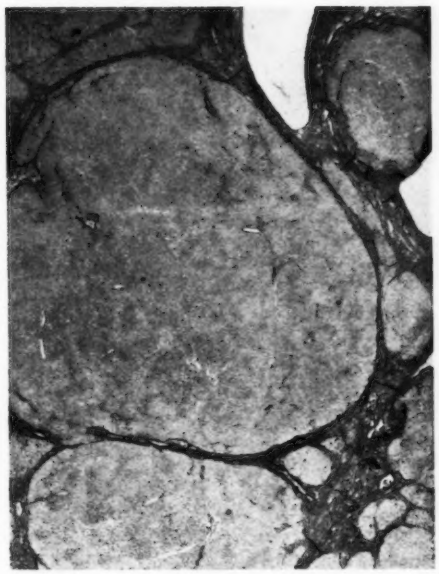
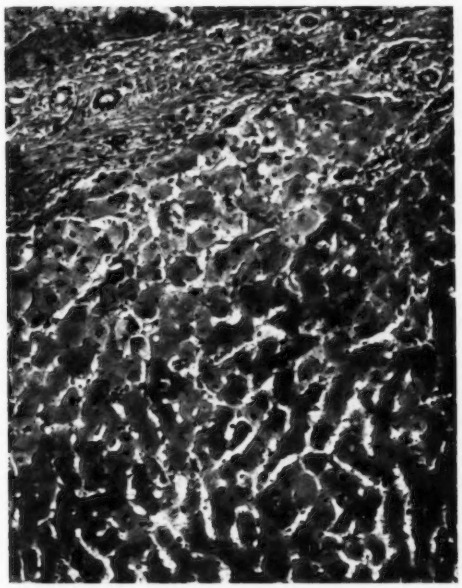
FIG. A. LIVER HISTOLOGY IN CASE OF WILSON'S DISEASE



a



b

While there is no characteristic lesion in this disease, the following changes are apparent:

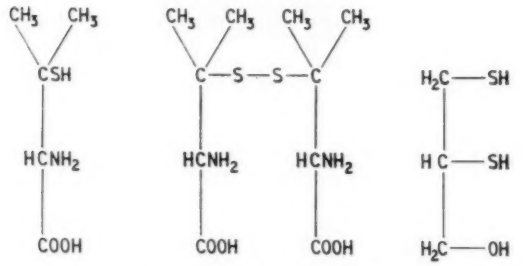
a: distortion of the lobular architecture

(Stain: silver, magnification × 8)

b: fibrous tissue and inflammatory cells at edge of large lobule shown in (a)

(Stain: haematoxylin and eosin; magnification × 150)

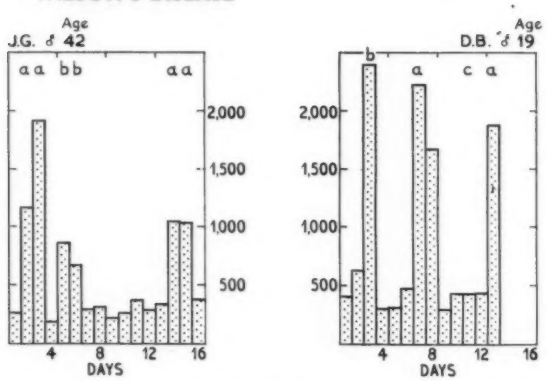
FIG. 1 CHEMICAL STRUCTURE OF SULPHYDRYL COMPOUNDS



ββββ′-Tetramethyl cystine

ββ-Dimethyl cysteine (Penicillamine) 2:3-Dimercaptopropanol (BAL)

FIG. 2. INCREASE IN URINARY EXCRETION OF COPPER AFTER ADMINISTRATION OF SULPHYDRYL COMPOUNDS TO TWO PATIENTS WITH WILSON'S DISEASE



The results are compared with those obtained by giving tetramethyl cystine.

Columns show excretion of urine copper (μg./24 hr.)

Letters at head of columns show type of administration, as follows:

a: 900 mg. penicillamine, given orally

b: 200 mg. BAL, given by intramuscular injection

c: 1.0 g. tetramethyl cystine, given orally

(1946) that 2:3-dimercaptopropanol (British Anti-Lewisite, BAL) (fig. 1) would mobilize copper from normal subjects, an observation that was later extended to one patient with Wilson's disease by Mandelbrote, Stanier, Thompson & Thurston (1948). Following this, BAL was used to treat four such patients by Cumings (1951) and five patients by Denny-Brown & Porter (1951). Two of Cumings' and all of Denny-Brown's patients showed a significant increase in copper excretion after BAL, and some clinical improvement. Other agents have since been shown to be effective in increasing the excretion of copper in the urine, intravenous "versene" (2–3 g. daily) being the most effective of these, but intravenous amino acids, cortisone and an increase in the dietary protein are also of value. Cartwright *et al.* (1954) found that the greatest increase in urinary copper could be achieved by giving a combination of intramuscular BAL and intravenous amino acids. As a long-term regime for treatment of patients with Wilson's disease, he recommended a high-protein diet, potassium sulphide (20 mg.) after meals to reduce the absorption of copper from the gut, and repeated courses of intramuscular BAL (200 mg. daily), a 5-day course being given every 10 days for life. However, he pointed out that BAL is an extremely painful injection and often gives rise to severe urticaria, pyrexial reactions or nausea and vomiting. More recently Bearn (1956), summarizing his earlier studies (1953) and those with Kunkel (1952, 1954), has stated that, to obtain the best results, BAL should be given in 300-mg. doses twice daily for months or years and that patients who can tolerate this treatment may show remarkable and continued improvement. Unfortunately he found some patients unresponsive to BAL; these usually had the more acute form of the disease. He also recommended the use of a low-copper diet, and sulphide or a carbo-resin to reduce copper absorption from the gut. Like Cartwright and his group, he found severe toxic reactions to the drug greatly limited its usefulness. Recently it has been shown that the sulphydryl amino acid penicillamine (dimethyl cysteine) (fig. 1) is of value in removing copper from patients with Wilson's disease (Walshe, 1956a). In a later and more detailed study (Walshe, 1956b) it was shown that penicillamine, in doses of 0.5–1.5 g. by mouth daily, produced a tenfold increase in the excretion of copper in two normal subjects as well as in six patients with Wilson's disease. In five of these patients the excretion following penicillamine was greater than that produced by the administration of BAL. Figure 2 shows the results from two of the patients investigated. A number of other compounds which it was thought might release sulphydryl groups in the body were also studied, but were all found to be inactive. That it was the —SH group and not the amino group on penicillamine that was the active radicle was shown by giving tetramethyl cystine (formed by the oxidation of dimethyl cysteine, cf. fig. 1) to one of the patients; but this, like the other compounds studied, did not give rise to any increase in the excretion of copper.

It has not as yet been possible to make a full assessment of the usefulness of this new form of treatment by a long-term clinical trial but, on theoretical grounds, these early results are very encouraging and may well indicate that penicillamine will be a valuable advance in the treatment of Wilson's disease.

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LIVER FAILURE

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1 Jaundice
2 Neuropsychiatric changes
  a Clinical features
  b The concept of portal-systemic encephalopathy
  c Acute and chronic hepatic coma
  d Treatment
3 Fetor hepaticus
4 Ascites
  a Aetiology
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5 Terminal electrolyte and circulatory changes
6 Changes in haemostasis
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Liver failure must be considered as a clinical syndrome, for it is difficult to equate specific functions of the liver cell with clinical findings. For instance, ascites, one of the most important associations of liver failure, cannot be related only to hepatocellular function, and liver coma is not due only to disease of the liver cells. Moreover, pure hepatocellular failure is extremely rare and there are usually additional features due to disordered intra-hepatic circulation, to intra- or extra-hepatic bile duct obstruction or to the reticulo-endothelial reaction which follows liver injury.

1. Jaundice

Jaundice may indicate failure of the liver cells to conjugate bilirubin with glucuronic acid (Billing & Lathe, 1956), intra-hepatic or extra-hepatic bile duct obstruction, or increased destruction of erythrocytes (Chaplin & Mollison, 1953). In any one patient the jaundice may be due to a combination of these factors. The jaundice of liver failure can often be reduced by adrenocorticotrophic hormone (ACTH) or cortisone (Sborov, Bluemle, Neefe & György, 1955). This therapy, however, does not affect the underlying disease process.

2. Neuropsychiatric Changes

a. Clinical Features

A neurological syndrome of the same basic pattern may complicate liver disease of varied aetiology, and the term hepatic coma encompasses a whole clinical syndrome of which coma is only a small part (Adams & Foley, 1953; Sherlock, Summerskill, White & Phear, 1954).

The lapse into coma may be abrupt, but more often mental confusion and apathy, or sometimes extreme emotional lability, is the first sign of deterioration (Davidson & Summerskill, 1956). The episodes may terminate at any stage, the patients having no recollection of their actions. Variation from day to day and even from hour to hour is particularly noticeable.

The most characteristic neurological sign is the " flapping " tremor best demonstrated with the patient's arms outstretched and fingers separated. It disappears in repose or in remission and is liable to be missed if the patient is only observed at rest. The flapping tremor also occurs sometimes in patients with uraemia or respiratory failure. Less specific findings include increased tendon reflexes and muscle tone, with ankle clonus and normal plantar responses. As coma deepens, muscle tone becomes flaccid, tendon reflexes can no longer be elicited and plantar responses become extensor. The diffuse nature of the cerebral disturbance is further shown by reversed sleep rhythm, grasping and sucking reflexes, and disorders of speech and vision.

The electroencephalogram (EEG) may show bilaterally synchronous slow waves of two-per-second frequency in the delta range. In the early stages these occur in bursts usually separated by the normal cortical rhythm (Foley, Watson & Adams, 1950). If the patient recovers, the EEG returns to normal. These EEG changes are occasionally found with other metabolic abnormalities of the brain, and are not specific for hepatic coma.

b. The Concept of Portal-Systemic Encephalopathy

The essentially reversible cerebral disturbance and the diffuse involvement suggest that the changes are metabolic. Similar nervous disorders have been induced in some patients with liver disease by oral administration of a high-protein diet, ammonium chloride, urea, methionine or choline (Van Caulaert, Deviller & Halff, 1932; Kirk, 1936; Phillips, Schwartz, Gabuzda & Davidson, 1952; Phear, Ruebner, Sherlock & Summerskill, 1956). This suggests that the material responsible is nitrogenous. Ammonium has been incriminated as the possible toxic substance. Theoretically, ammonium might interfere with cerebral metabolism. Bessman & Bessman (1955) showed that in hepatic coma there is an increased arterial–jugular bulb ammonium difference, indicating ammonium utilization by the brain. Of the many reactions utilizing ammonia in the body, two are of significance in the brain—glutamine synthesis and the reversal of glutamate oxidation, namely reductive amination of α-ketoglutarate. Synthesis of glutamate from α-ketoglutarate could produce the phenomena seen in hepatic coma by interfering with the Krebs cycle[1] which is the most likely final oxidative pathway in the brain. The diminished cerebral oxygen metabolism in hepatic coma (Wechsler, Crum & Roth, 1954) would agree with this hypothesis. In contrast, however, Webster & Davidson (1956a) report that the brain in hepatic coma does not always take up ammonia; but occasionally the jugular bulb value may exceed that in an artery. The blood-ammonium level is frequently, although not constantly, raised in patients in hepatic coma (Phear, Sherlock & Summerskill, 1955) (see also fig. 1). Identical neurological changes can be precipitated by oral methionine without producing a constant rise in the blood-ammonium level (Phear *et al.* 1956). The relation of blood-ammonium levels to liver coma might thus be similar to the relation of blood-urea levels to clinical uraemia (Singh, Barclay & Cooke, 1954): in both cases a correlation can be established but in neither is the relationship causal.

Although there is speculation concerning the nature of the toxic substance(s), it is probably of intestinal origin. Observations have shown two routes by which toxic substances of

[1] See Krebs ,*Brit. med. Bull.* 1953, 9, 97.—ED.

intestinal origin might reach the systemic circulation in patients with liver disease. In patients with severe hepatocellular disease (e.g. severe virus hepatitis), the failing liver allows the substances to pass unaltered into the peripheral blood (see fig. 2). In patients with cirrhosis, in addition to impaired liver function, the presence of a collateral circulation delivers portal vein blood directly to the peripheral blood stream before it can be metabolized by the liver (see fig. 3). The neurological syndrome can therefore be termed a portal-systemic encephalopathy (Sherlock *et al.* 1954; White, Phear, Summerskill & Sherlock, 1955). The development of the syndrome in a patient with cirrhosis depends upon the balance between the extent of the collateral circulation and the capacity of the liver to metabolize toxic factors (see fig. 4).

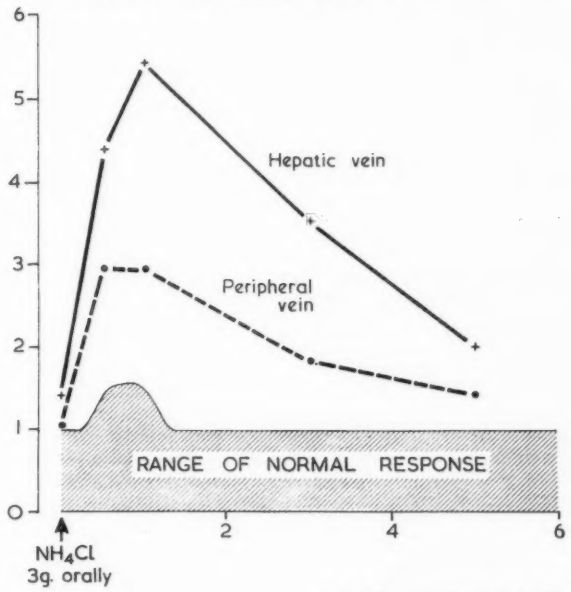
c. Acute and Chronic Hepatic Coma

The syndrome may be classified into an acute type, with or without an additional precipitating factor, and a chronic type.

The acute type is best seen in fulminant virus hepatitis. In cirrhosis, the neuropsychiatric changes may occur spontaneously in the terminal stages. Factors precipitating the acute type in cirrhosis do so either by impairing liver function or by increasing the amount of toxic nitrogenous substance in the intestine. Gastrointestinal haemorrhage—usually from oesophageal varices—is the equivalent of a large protein meal, and the hypotension and anaemia impair hepatic oxygen supply. Other precipitants are infections, alcoholism and drugs, especially morphia and barbiturates. Coma may also follow a large protein meal or therapeutic doses of ammonium chloride, methionine or choline.

Paracentesis abdominis may also initiate coma, although

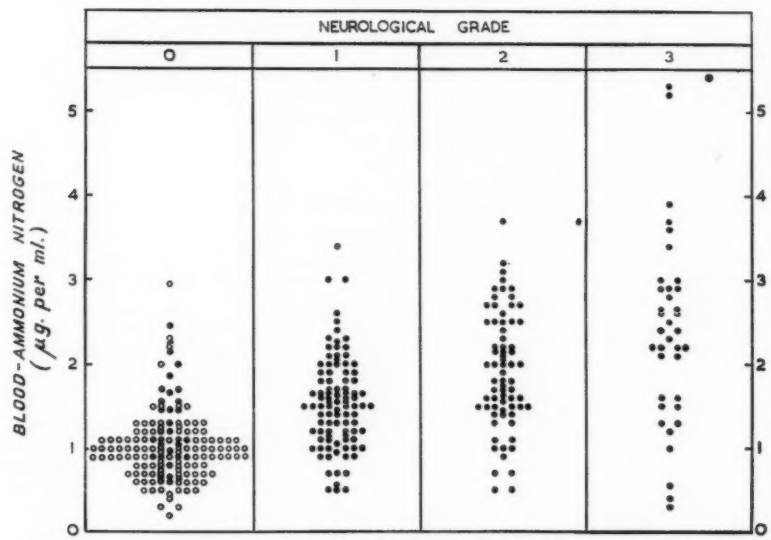
FIG. 2. SIMULTANEOUS SAMPLES FROM PERIPHERAL AND HEPATIC VEINS SHOWING ABNORMAL BLOOD-AMMONIUM CURVES FOLLOWING ADMINISTRATION OF 3 g. AMMONIUM CHLORIDE BY MOUTH IN A PATIENT WITH SEVERE INFECTIVE HEPATITIS (female, age 52)



Sherlock, Summerskill, White & Phear (1954) by permission of the Lancet

Abscissae: time (hr.)
Ordinates: blood ammonium (μg./ml.)

FIG. 1. BLOOD-AMMONIUM LEVELS RELATED TO GRADE OF NEUROLOGICAL DISABILITY



Phear, Sherlock & Summerskill (1955) by permission of the Lancet

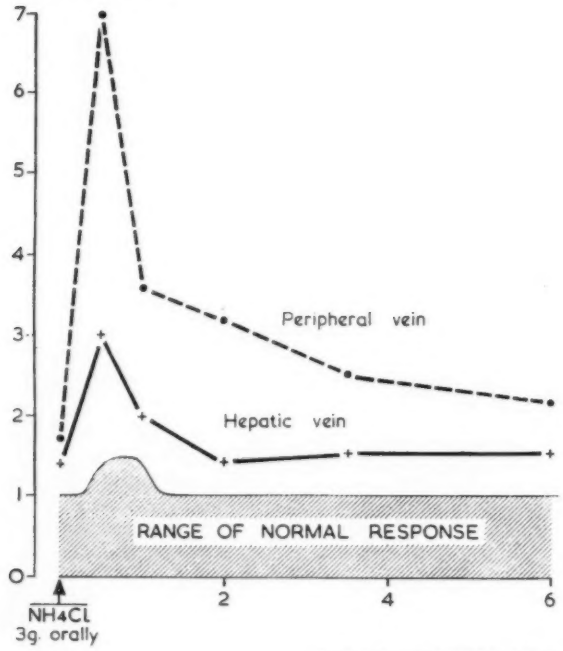
Solid circles: neurological disability present or past
Open circles: no neurological signs
The neurological status was graded as 0 (normal), 1 (minor disorder of consciousness and the motor system), 2 (gross disorder of consciousness and the motor system), or 3 (coma).

the mechanism is uncertain. Electrolyte imbalance following the withdrawal of large quantities of sodium and water, changing hepatic circulation when portal-vein pressure is reduced, and hypotension due to a vaso-vagal reaction may contribute.

The carbonic anhydrase inhibitor acetazolamide (Diamox), given for some days, occasionally causes neuropsychiatric changes in the patient with cirrhosis (Kunkel, unpublished observations, 1952), and there is an associated increase in blood-ammonium levels (Webster & Davidson, 1956b). Diamox impairs ammonium tolerance in liver disease and diminishes the ammonium uptake by peripheral tissues (de Groote, Dawson, Rosenthal & Sherlock, 1957).

The chronic type is associated with an extensive collateral circulation (Summerskill, Davidson, Sherlock & Steiner, 1956). This may be demonstrated only by transsplenic portal venography (Atkinson, Barnett, Sherlock & Steiner, 1955). This circulation may follow thrombosis of the portal vein; sometimes the umbilical vein is widely patent or the collateral circulation

FIG. 3. BLOOD-AMMONIUM CURVES FOLLOWING ADMINISTRATION OF 3 g. AMMONIUM CHLORIDE BY MOUTH IN A PATIENT WITH CIRRHOSIS OF THE LIVER, PORTAL VEIN THROMBOSIS AND AN EXTENSIVE PORTAL COLLATERAL CIRCULATION (male, age 59)



Sherlock, Summerskill, White & Phear (1954) by permission of the Lancet

Abscissae: time (hr.)
Ordinates: blood ammonium (μg./ml.)

may be extensive, without a single major vessel. A surgical porta-caval anastomosis is followed by chronic neuro-psychiatric symptoms in 10–15% of patients (McDermott & Adams, 1954; Riddell, 1955).

The portal collateral circulation is so important in this group that it may be questioned whether it should be described under the heading of liver failure. However, there is always some liver disease in addition. The intermittent neuropsychiatric disturbance is very chronic, continuing for as long as six years (Summerskill *et al.* 1956). The patient may be seen for the first time in coma or in remission, adding to the diagnostic difficulty. These patients are particularly sensitive to changes in dietary protein.

d. Treatment

All dietary protein is stopped and at least 1,600 calories are supplied daily as glucose drinks or as intragastric or intravenous glucose. Potassium supplements are often needed when glucose is the only source of calories. During recovery, protein is added in 20-g. increments on alternate days. Patients with an acute episode of coma soon achieve a normal dietary protein intake. In the group with chronic disease, permanent protein restriction is needed to control mental symptoms (Summerskill *et al.* 1956); the limit of tolerance is usually 40–50 g. protein per day. Supplements of vitamin K and of B complex are given parenterally as a matter of routine.

An enema is given and this is repeated if necessary. Oral chlortetracycline protects patients with liver disease from the adverse neurological effects of oral DL-methionine (Phear *et al.* 1956). The bacterial flora of the small gut is increased in patients with hepatic cirrhosis, and the number of organisms and their effect on methionine are much reduced by the antibiotic (Martini, Phear, Ruebner & Sherlock, 1957). Oral chlortetracycline is therefore given (if necessary by a stomach tube), a loading dose of 2 g. being followed by 5 g. 6-hourly for the duration of symptoms or to a maximum of one week. Diarrhoea may complicate chlortetracycline therapy, and long-term use may be associated with the emergence of resistant organisms. Neomycin may well be the more satisfactory antibiotic (Fisher & Faloon, 1956) although, at the present time, the expense is prohibitive.

Patients in impending coma are extremely sensitive to sedatives. If the patient is uncontrollable, half the usual dose of pentobarbital may be given. Morphia is absolutely contraindicated. Drugs known to induce hepatic coma such as ammonium salts, ammonium-exchange resins, methionine, urea and Diamox are disallowed.

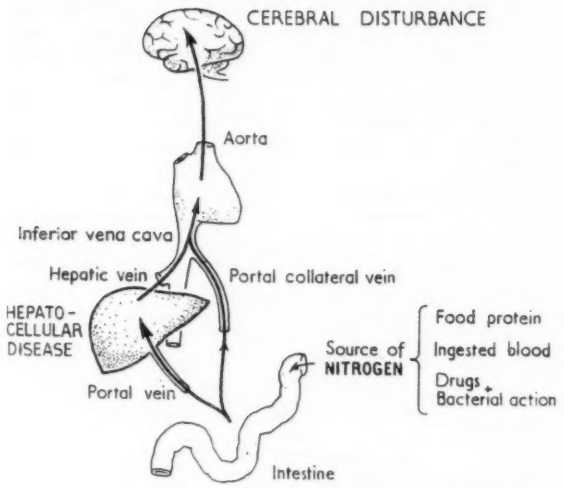
Gastrointestinal haemorrhage is energetically treated. In association with hepatic coma it is always an indication for use of Sengstaken oesophageal compression tube. Blood transfusion may need to be prolonged. Toxic factors, including significant and increasing amounts of ammonium, develop rapidly in blood after shedding and, where possible, fresh blood is used.

Glutamic acid therapy is based on the supposition that ammonium toxicity is important in the genesis of hepatic coma. Glutamic acid should react with the ammonium-forming innocuous glutamine (Walshe, 1953). Good results have been claimed in small series (Walshe, 1953; Priest, Whitehead & Whittaker, 1953) although this has not been confirmed by wider application under controlled conditions (Sherlock *et al.* 1954; Alexander, Berman & Balfour, 1955). Although the blood-ammonium level may fall after glutamic acid, there is little clinical improvement (Sherlock, Summerskill & Dawson, 1956).

Corticoid hormones are not recommended for hepatic coma occurring in patients with cirrhosis. In patients where the syndrome complicates virus hepatitis, recovery is so rare that cortisone in very large doses such as 1,000 mg. daily (Ducci & Katz, 1956), or preferably prednisolone, may be tried.

It is dangerous to give amino acids such as methionine which are toxic to these patients. Amino acids cannot be metabolized by the failing liver and are already being excreted in the urine. Similarly lipotropes such as choline prove of no value and may be toxic. In one series of 66 patients treated by the conservative regime 47 reached deep coma, and 39 (58%) recovered (Sherlock *et al.* 1956). Assessment of therapeutic results in hepatic coma is complicated by the unpredictable fluctuations in clinical course. Prognosis depends on the extent of liver-cell failure. The chronic-disease group with relatively good liver function but with an extensive collateral circulation combined with increased intestinal nitrogen has the best prognosis, and the acute-hepatitis group the worst. In cirrhosis the outlook is poor if the patient has

FIG. 4. THE MECHANISM OF NEUROLOGICAL CHANGES LEADING TO HEPATIC COMA



Sherlock, Summerskill, White & Phear (1954) by permission of the Lancet

The three important factors are:

1: nitrogenous substances in the intestines
2: portal-systemic collateral veins diverting blood past the liver
3: depressed hepatocellular function

ascites, jaundice and a low serum-albumin level, all indicative of poor liver-cell function.

Altered liver function allowing the passage of toxic nitrogenous substances from the intestine to the brain is only one aspect of hepatic coma or indeed of liver failure. The regime described cannot control the electrolyte disturbance and other unrecognized metabolic changes which must be occurring when the liver fails. Moreover, no treatment directed solely towards correction of altered cerebral metabolism can be expected to succeed constantly in these circumstances. The conservative regime suggested seems the best available and will be modified when other effective remedies are discovered.

3. Fetor Hepaticus

This is a sweetish, slightly faecal smell of the breath and has been likened to that of a freshly opened corpse (Himsworth, 1947) or mice. It occurs in patients with severe hepatocellular disease and also with an extensive collateral circulation. It is presumably of intestinal origin, for it becomes less intense after defaecation or when the gut flora is changed by wide-spectrum antibiotics. Challenger & Walshe (1955) found methyl mercaptan in the urine of a patient with hepatic coma who exhibited fetor hepaticus. This substance can be exhaled in the breath, and might be derived from methionine, the normal demethylating process being inhibited by liver damage.

In patients with acute liver disease, fetor hepaticus is a bad omen and often precedes coma. In patients with extensive portal collaterals, it may occur transiently and may not be such a grave sign. Fetor may be a useful diagnostic sign in patients seen for the first time in coma.

4. Ascites

a. Aetiology

Patients with liver disease developing ascites retain sodium avidly (Eisenmenger, Blondheim, Bongiovanni & Kunkel, 1950; Ricketts, Eichelberger & Kirsner, 1951). This is partly related to the Starling hypothesis that the interchange of fluid between the blood and the tissue spaces is controlled by the balance of the capillary blood pressure forcing fluid into the tissue spaces, and the difference between osmotic pressure in the vascular and extravascular compartments retaining fluid in the vascular compartment. In cirrhosis, the osmotic pressure of the plasma proteins falls owing to the failure of the liver to manufacture albumin, and the intra-hepatic vascular distortion is associated with portal-venous hypertension. These are not the only mechanisms, for increased amounts of the salt-retaining hormones of the adrenal cortex (aldosterone) have been found in the urine of patients with cirrhosis-forming ascites (Axelrad, Cates, Johnson & Luetscher, 1955; Wolff, Koczorek, Jesch & Buchborn, 1956). The increased amounts might be due to hepatic functional impairment with failure to detoxicate the hormone, or to over-production. In man, bilateral adrenalectomy has been performed in a patient with cirrhosis and intractable ascites. Urinary sodium increased and the response to mercurial diuretics was improved, although ascites persisted and dietary therapy with diuretics and exchange resins was still necessary (Marson, 1954).

The part played by the antidiuretic hormone (ADH) is probably small, for the ADH activity of urine in cirrhosis is normal (Stein, Schwartz & Mirsky, 1954) and the cirrhotic liver can dispose of ADH normally (White, Rubin & Leiter, 1951).

Oestrogens have a salt-retaining action in animals (Thorn & Engel, 1938), and are detoxicated by the liver (Pearlman, 1948). Administration of oestradiol benzoate to patients with cirrhosis and ascites causes retention of sodium chloride and water (Preedy & Aitken, 1956), and this may be yet another mechanism contributing to the fluid retention.

b. Treatment[2]

Raising plasma osmotic pressure, for instance by human albumin infusions, is of only transitory benefit. It is not safe in the presence of ascites to lower the portal-vein pressure by surgery. There is, however, also the intense sodium retention. Virtually all the sodium taken with food is retained with water to form ascites and, on an unrestricted intake, repeated paracenteses are necessary. Each tap results in loss to the body of the protein contained in the ascitic fluid and, as ascites reaccumulates, this is made up with difficulty by protein manufactured by the failing liver. The patient shows progressive loss of flesh. Reaccumulation of ascites can be prevented by rigid restriction of dietary sodium (22 m-equiv. = 0.5 g. sodium per day). The drain of protein into the ascites is stopped and serum albumin rises until it reaches levels so high that ascites formation ceases. These patients benefit from a high-protein diet, which is reduced only with impending coma. Most natural protein foods contain much salt so that, to achieve a satisfactory intake, salt-poor protein supplements such as Casilan must be used. Mercurial diuretics are given twice weekly with ammonium chloride (3 g. per day) and potassium chloride (1 g. per day). It is unusual for

[2] See also Stokes & Rosenheim, p. 142 of this number of the Bulletin.—Ed.

this small dose of ammonium chloride to precipitate hepatic coma.

As nutrition improves there is a rise in the serum-albumin level until the production of ascites ceases, and it may be possible to relax sodium restriction. Therapeutic failures are usually due to the severity of the hepatocellular failure, to the patient's entering the terminal stage of hyponatraemia, to lack of co-operation, or to the presence of a complicating liver-cell cancer.

5. Terminal Electrolyte and Circulatory Changes

In the terminal stages of liver failure, ascites, if present, fails to respond to the above regime, urine output diminishes, jaundice deepens, the blood pressure falls and the neuropsychiatric changes are constant. Blood urea rises and the serum-sodium level falls; low serum-sodium values are usual in cirrhotic patients with ascites and oedema, values below 130 m-equiv./l. are ominous, and recovery is most unusual when levels fall below 125 m-equiv./l.

The mechanism of the hyponatraemia is very complex. A comparison has been made with the " low-salt syndrome ". This condition is caused by excessive sodium restriction with depletion of body-sodium by diarrhoea, vomiting, diuretics, exchange resins or removal of oedema or ascitic fluid. Relief, with diuresis and rise in blood pressure, follows intravenous hypertonic saline. Four patients with the hyponatraemia of liver failure were given saline therapy. Given unrestricted water, the additional sodium was completely retained in isotonic dilution, with further increase of an already expanded extracellular volume. In two patients the hypertonic saline was followed by pulmonary oedema and even seemed to accelerate death (Hecker & Sherlock, 1956). Sodium should not be given to patients in liver failure unless there is clear evidence that profound sodium loss has been sustained.

The apparent hyponatraemia might be due to redistribution of sodium, a relatively greater fraction occurring in the intracellular compartment (Talso, Strub & Kirsner, 1956). This concept has not been confirmed by actual measurements of sodium in the body compartments. The term " dilution hyponatraemia " should therefore be avoided, since it is uncertain how much the low serum-sodium value is due to an expanded extracellular space (dilution) and how much to redistribution of sodium between extracellular and intracellular fluids. The hyponatraemia could be effected either by retention of water or by excretion of sodium chloride. Patients in terminal liver disease are certainly not excreting sodium and must therefore be retaining water. This might be the result of antidiuretic hormone, the osmo-receptors controlling its output being set at a lower level, although there is little evidence that this is so. Aldosterone must also be considered, but this has little effect on water metabolism.

The azotaemia seems unrelated to organic changes in the kidney. It might be associated with the hypotension and renal circulatory failure and with the presence of blood in the intestine. This, however, cannot be the whole explanation, for blood urea may be raised before the development of either hypotension or gastrointestinal haemorrhage.

Warm dry hands, hypotension, and a soft full pulse are common features of decompensated liver disease, and the cardiac output is increased in patients with cirrhosis of the liver (Kowalski, Abelmann & McNeely, 1954). Peripheral venous blood is highly oxygenated (Silverstein, 1956). The hyperdynamic circulation is not solely due to chronic anaemia. The low blood pressure with increased cardiac output suggests vasodilatation, but the mechanism of this is uncertain. In one patient, the low oxygen saturation of hepatic venous blood suggested a low hepatic blood flow (Hecker & Sherlock, 1956). It is known that the liver does not share in the increased cardiac output of other hyperdynamic states such as thyrotoxicosis (Myers, Brannon & Holland, 1950). Indeed, in the presence of hypotension, liver blood flow is reduced and this may contribute to the liver failure. This is the rationale for raising systemic blood pressure. Noradrenaline is a peripheral constrictor with little effect either on the splanchnic circulation or on the cardiac output (Bearn, Billing & Sherlock, 1951). This substance may have a dramatic temporary effect in improving the state of consciousness and increasing urinary volume and is worth trial (Hecker & Sherlock, 1956). Blood transfusion may be similarly effective.

6. Changes in Haemostasis

The bleeding tendency in liver failure is of multiple causation. The *thrombocytopenia* associated with reticulo-endothelial hyperplasia and splenomegaly is contributory, but there is also a profound disturbance of many other haemostatic factors. Some of these are proteins whose synthesis is, presumably, difficult when liver-cell function fails. *Prothrombin* migrates electrophoretically with the serum albumin fraction and is diminished in liver-cell disease when serum albumin levels are low. In contrast to the hypoprothrombinaemia of obstructive jaundice, this deficiency is not corrected by parenteral vitamin K therapy. Deficiency of *Factor V*, a globulin concerned with thromboplastin regeneration, may contribute to the bleeding tendency and prolonged one-stage " prothrombin " time.

Christmas factor is also concerned with thromboplastin regeneration and may be deficient in patients with parenchymal liver disease (Cowling, 1956). This is not revealed by either the one-stage or the two-stage prothrombin times.

Fibrinogen is manufactured in the liver, but its deficiency is most unusual even in severe liver failure. Increased *fibrinolytic activity* of blood may also be found in patients with cirrhosis of the liver (Kwaan, McFadzean & Cook, 1956; Fearnley, 1956).

The two-stage prothrombin time test should be employed in all patients with liver-cell failure. The one-stage method is not so useful, being influenced only to a small extent by prothrombin lack. It may be within normal limits in the presence of prothrombin deficiency.[3]

Vitamin K, parenterally, should be given routinely. It is innocuous, and although the coagulation defect of parenchymatous liver disease will not be affected, deficit of prothrombin and Factor VII due to any obstructive element will be corrected.

If Christmas factor is believed lacking, serum should be given. Fresh blood transfusion is given for the profound bleeding diathesis of terminal liver failure.

[3] See Biggs, *Brit. med. Bull.* 1955, **11**, 5.—ED.

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TREATMENT IN DIFFUSE LIVER DISEASE

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1 Acute hepatitis
2 Post-hepatitis syndrome
3 Subacute hepatitis
4 Biliary obstruction
5 Cirrhosis
  a Ascites
  b Hypersplenism
  c Portal hypertension
  d Coma
6 Diet in liver disease
7 Conclusion
References

There have been few dramatic advances in the treatment of diffuse liver disease during the last decade, and therapy remains symptomatic rather than specific. Critical evaluation of the nature of liver disease has, however, led to more accurate definition of certain symptom-complexes occurring either singly or together: portal hypertension, for instance, requires assessing in cirrhosis independent of hepatic failure; the depth of hepatic coma must not be accepted as a measure of severity of hepatocellular damage, but may depend on the extent of portal-systemic anastomoses. A clearer understanding of these symptom-complexes provides a foundation for more rational treatment. Whereas it may not be possible to lay down hard and fast rules for treatment of a " case of cirrhosis ", it is not so difficult to make a therapeutic plan for a " man aged 50 with cirrhosis, ascites and hypersplenism, but without gastrointestinal haemorrhage or coma ".

By consideration of these different aspects of diffuse liver disease, it should be possible to lay down firmly the principles of dietary modification best suited to each individual case; to control ascites without provoking further symptoms of a different order; and to arrive at some tentative conclusions as to the present position of cortisone, corticotrophin and allied substances in treatment; to assess the part which the surgeon has to play, and to point the dangers and drawbacks of some therapeutic substances commonly used in the treatment of hepatitis in its various stages.

1. Acute Hepatitis

Acute hepatitis has a natural tendency towards complete recovery. The mortality rate is low and progress to subacute hepatitis rare. In the absence of any specific antiviral remedy, treatment is mainly symptomatic though aimed at shortening the clinical course.

Rest. Rest is continued after the acute symptoms have subsided in order to reduce the chances of relapse. The minimum period is desirable in order to avoid a post-hepatitis anxiety state, and to cause as little financial embarrassment as possible for the working patient. Orthodox treatment demands rest in bed until appetite has returned, the liver is no longer tender, the morning urine is free of urobilin, and serum bilirubin is less than 1.5 mg./100 ml.

Chalmers and his associates (Chalmers, Eckhardt, Reynolds, Cigarroa, Deane, Reifenstein, Smith & Davidson, 1955), drawing conclusions from controlled observation of American soldiers suffering from acute infective hepatitis, have stressed two points:

i. that physical activity to the point of fatigue in the acute phase is harmful, and rest in bed must be undertaken at once;

ii. that no harm is done by mobilization as soon as the patient feels well, irrespective of the depth of jaundice.

They found no increased rate of relapse in soldiers sent out of hospital when the serum bilirubin was greater than 1.5 mg./100 ml. and bromsulphalein retention greater than 6% in 45 minutes. This finding is contrary to the generally accepted view and, as has been stressed (*Lancet*, 1956), their sample of healthy young soldiers is not representative of the incidence of hepatitis in civilians. Caution should still be observed in most cases, especially in the presence of other disease processes.

Diet. Animal experiments and observation of human liver disease in countries whose inhabitants live largely on cereals suggest that a low-protein, high-carbohydrate diet is injurious to the liver. This has resulted in the traditional high-protein diet for the sufferer from acute hepatitis. Anorexia limits its acceptance in the early stages, but patients will eat voraciously once appetite has returned. Most will be content with 100 g. protein per day, and the advantages of a higher protein content are not clear (Darmady, 1945; Davidson, 1955; Hendrix, 1955) though Chalmers *et al.* (1955) have recommended 150 g. protein in a 3,000-calorie diet.

There is no need for amino-acid supplements at any time. Choline is unnecessary (Richardson & Suffern, 1945) and unpleasant to take; methionine is potentially dangerous since it may provoke coma (Phillips, Schwarz, Gabuzda & Davidson, 1952; Sherlock, Summerskill, White & Phear, 1954).

The carbohydrate intake can be varied within wide limits without influencing the progress of the disease, and there is no evidence that either a high-fat or a low-fat diet is detrimental. The amount of fat in the diet may be dictated by the fancy of the patient.

Drugs. The prothrombin concentration may fall in acute hepatitis, either owing to non-absorption of vitamin K in deeply jaundiced patients or to hepatocellular failure in a severe infection. Intramuscular injection of menaphthone esters such as Synkavit or intravenous injection of vitamin K oxide daily is appropriate treatment.

Nausea may be relieved by Avomine but it must be remembered that this drug often induces drowsiness which may be falsely interpreted as a sign of impending coma. Dyspepsia may require magnesium trisilicate, and constipation should be avoided by the use of magnesium sulphate which has some action as a cholagogue.

Depression in acute hepatitis, as in many viral diseases, may be extreme. Attempts to relieve it by using chlorpromazine are unwise, since this drug may produce jaundice, irrespective of present or past liver damage, by causing widespread obstruction of the small bile channels (Loftus, Huizenga, Stantler & Rome, 1955; Stacey, Azima, Huestis, Howlett & Hoffman, 1955; Murphy & Ofner, 1956).

We believe that cortisone has no place in the treatment of uncomplicated acute hepatitis. In fact, relapse has been reported to be more frequent after its use (Evans, Spring & Nelson, 1953). Huber & Wiley (1955) are, however, enthusiastic as a result of a trial on 100 cases of acute hepatitis, finding that jaundice went more quickly, weight increased more quickly, the patients "got well" more quickly, and the bromsulphalein retention returned to normal more quickly than in their control group. Hendrix (1955) considers the only indications for cortisone and adrenocorticotrophic hormone to be prolonged anorexia, persistent obstruction and desperation. The occasional report of a case of fulminant hepatitis whose recovery is attributed to cortisone (e.g. Ducci & Katz, 1952; Shane, Gamm & Gamm, 1955) cannot be properly evaluated, since other treatments are always applied to a patient in such dire straits, and the comparative rarity of the circumstances does not allow of a controlled series.

2. Post-Hepatitis Syndrome

This consists of prolonged or recurrent fatigue, accompanied by anorexia, vague upper abdominal discomfort, usually right-sided, together with fat intolerance. There is usually a tender palpable liver. These symptoms may be severe enough to demand occasional days off work, but are not accompanied by any biochemical or histological signs of hepatocellular failure other than positive flocculation tests, and do not indicate the presence of subacute hepatitis. They require no special treatment other than reassurance and do not usually persist for more than 18 months. It should be noted that flocculation tests may remain abnormal for some months after clinical recovery from hepatitis, and are therefore an unsatisfactory guide to prognosis.

This syndrome should be distinguished from a post-hepatitis anxiety state, in which more usual symptoms of anxiety may be found, together with such signs as tachycardia and brisk tendon reflexes.

Another symptom that may follow hepatitis is alcohol intolerance, which is unrelated either to a true post-hepatitis syndrome or to anxiety. The patient remains perfectly well so long as he avoids alcohol, the taking of which results in nausea, anorexia, and vague upper abdominal discomfort. The treatment is abstention and the intolerance disappears, as a rule, in 18 months. This intolerance is probably the basis for the common advice to avoid all alcohol for six months in convalescence. In our view an occasional glass of wine or sherry is not normally contra-indicated.

3. Subacute Hepatitis

Though the definition of subacute hepatitis rests on non-recovery from an acute attack, the onset is often insidious. It is marked by anorexia, nausea, jaundice and, maybe, persistent fever. The liver is enlarged, firmer than normal, and tender. Liver function tests remain abnormal and histology of a biopsy specimen indicates the presence of necrosis, fibrosis and regeneration in a liver whose reticular architecture is deranged.

Cortisone may be of some value in these cases. By suppressing inflammatory reaction it may reduce fibrosis and also limit the depth of jaundice, which depends in part on intra-hepatic obstruction. If steroid therapy is begun, a prolonged course is advisable. Prednisone and prednisolone, which cause less sodium retention, may prove to be the best drugs to use.

4. Biliary Obstruction

Difficulty often arises in distinguishing subacute hepatitis from extra-hepatic biliary obstruction. While the various liver function tests often resolve this problem, they are on occasion unhelpful.

It is, for instance, easy to overlook the presence of a carcinoma of the ampulla of Vater, and in cases of doubt laparotomy is always justified. On the other hand, it must be remembered that symptomless gallstones are often found in patients with cirrhosis of the liver. If biliary obstruction is suspected and the cause is not readily apparent, exploration of the bile duct and cholangiography are required.

Biliary obstruction is often complicated by cholangio-hepatitis with fever, rigors and pruritus (Himsworth, 1950). The infection in the biliary tract may respond to sulphadimidine or one of the antibiotics, but is unfortunately liable to recur.

Pruritus may be controlled in its milder forms by local applications of calamine and phenol, but these usually fail. Antihistamines, either parenterally or locally, may be more effective. Intractable cases should be given a trial of methyl testosterone (Ahrens, Payne, Kunkel, Eisenmenger & Blondheim, 1950; Lloyd-Thomas & Sherlock, 1952). It must be remembered that methyl testosterone may increase the depth of jaundice, and it is therefore wise to delay giving it until it has been definitely decided whether the obstruction to the biliary tract lies within or without the liver.

Surgery has no part as yet to play in the relief of intra-hepatic obstruction. Cholecystenterostomy, the use of polythene tubes, or reconstruction operations anastomosing the hepatic ducts to the jejunum, will be appropriate according to the nature and site of the extra-hepatic obstruction.

5. Cirrhosis

The aetiology of cirrhosis in its late stages is as difficult to determine as that of chronic nephritis. Both represent a well-defined syndrome resulting from scarring and parenchymal failure. It is usually stated that, in Britain, in the 50% of cirrhotic patients in whom any aetiological factor can be fairly established, cirrhosis in the majority follows acute hepatitis rather than a prolonged high-alcohol, low-protein intake. This view is based on analysis of hospital cases. In the experience of one of us, alcohol is a commoner cause in private patients. Other factors are of importance in the tropics (Waterlow & Bras, p. 107 of this number of the Bulletin). The aetiology of too many cases remains obscure, and we must be constantly on the watch for any potentially hepatotoxic substances which may bear some relation to this unsolved problem.

As with nephritis, it is improbable that any form of treatment can affect the established disease; but the complications of ascites, hypersplenism, portal hypertension and coma require discussion.

a. Ascites

While ascites occurs only rarely in patients with severe acute hepatitis, it is a common finding in cirrhosis of the liver. Its appearance is mainly determined by two factors, the

falling plasma albumin level found in liver failure and the raised portal venous pressure of portal hypertension. Ascites occasionally appears soon after a severe haemorrhage, probably as the result of haemodilution of the plasma, which lowers the plasma osmotic pressure, and it disappears when the blood loss has been made good, either naturally or by blood transfusion (Himsworth, 1950).

Unfortunately the surgical treatment of portal hypertension has only rarely resulted in relief of ascites. Until recently paracentesis was the main treatment, but such mechanical removal of fluid was often unsatisfactory because of rapid reaccumulation. Repeated tapping leads to a loss of valuable protein from the body and tends also to induce a low-sodium syndrome as the fluid reappears in a salt-depleted patient.[1]

With a clearer understanding of the role of salt and water retention and of the lowered plasma osmotic pressure, the management of ascites has become increasingly medical, depending largely on diet and diuretics. Sodium intake must be reduced to about 200 mg. per day, while efforts are made to increase its excretion. Diuretics such as mersalyl and acetazolamide (Diamox) are often of great value, but should be used with care, since sodium depletion and diminished plasma volume may occur even in the presence of ascites.

Ion-exchange resins can rarely be used, since the ammonium absorbed off the resin may lead to portal-systemic encephalopathy in the presence of hepatic failure and shunts. For similar reasons it may not be possible to maintain the patient on a high-protein diet, but if this can be tolerated it is often of considerable benefit. A low-sodium diet with 150 g. of protein per day is often of value in the prevention and treatment of ascites. With such a regime, and with blood transfusion when necessary, the outlook for the cirrhotic patient with ascites has been greatly improved.

b. Hypersplenism

It is not uncommon to find a reduction of all the formed elements of the blood in association with cirrhosis. This is probably due to splenic enlargement, as panhaemocytopenia occurs with many forms of splenomegaly. Attention is usually called to this by the presence of anaemia and its symptoms. Less often there is a positive capillary fragility test and sometimes frank purpura due to platelet deficiency. It is rare for leucopenia alone to be accompanied by symptoms.

A recognition of this syndrome of congestive splenomegaly with hypersplenism makes the retention of the term " Banti's syndrome " both unnecessary and undesirable.

Splenectomy is disappointing, since it rarely has any beneficial effect on the level of red cells, and is often followed by thrombosis of the splenic vein, thus prejudicing any later shunt operation.

Occasionally splenomegaly is associated with haemolytic anaemia, and in such cases splenectomy may be of benefit. No information appears yet to be available upon the effects of steroid therapy.

c. Portal Hypertension

The presence of portal hypertension may be inferred from the observation of dilated veins on the anterior abdominal wall, or from the demonstration of oesophageal varices radiologically or at oesophagoscopy. Splenic venography is of assistance in determining the site of obstruction to the portal system (see Sutton, p. 99 of this number of the Bulletin).

The fact of portal hypertension does not in itself demand treatment, since it is dangerous only by virtue of the bleeding engendered in the gastric and oesophageal varices, and these may be demonstrable many years before any such haemorrhage occurs. Once there has been one haemorrhage, however, serious consideration must always be given to the possibility of operative prophylaxis against a recurrence.

The control of bleeding from varices is of importance, not merely because of the blood loss but also on account of the consequent occurrence of ascites and coma. Expectant treatment with sedation and transfusion often suffices, though morphia must be given with care. If haemorrhage persists, the choice lies between direct surgical attack and control by a Sengstaken tube (Sengstaken & Blakemore, 1950). This is a triple tube, one part controlling a gastric balloon which is inflated and so exerts upward pressure on the gastric veins leading to the varices. This upward pressure produces pain and retching, and these features may be troublesome, even though they may be reduced by inflation of the oesophageal balloon controlled by the second part of the tube. The third part carries food to the stomach. Oesophageal ulceration and asphyxia due to laryngeal obstruction have been reported following the use of this tube (Bennett, Baker & Baker, 1952).

Surgical attack has been directed at the varices themselves (Macpherson, Owen & Innes, 1956) by gastric and oesophageal transection. Varices are likely to re-form after ligation, but it is easier to repeat an operative attack on them than it is to repair a clotted shunt. It is only operations such as these that have a part to play in the control of intractable bleeding (Linton, 1953), as they are more easily tolerated than a shunt by an ill patient.

Unfortunately few patients with cirrhosis are suitable cases for surgery in any form since, by the time portal hypertension has given rise to bleeding, liver function is always impaired to some degree, and both operative risk and immediate prognosis depend almost entirely on the state of hepatocellular function. Poor function implies a high operative mortality and also a short life (Reynell, 1951). It is in those cases of extra-hepatic portal obstruction or cirrhosis with well-preserved liver function that elective surgery is most useful (Milnes Walker, 1957).

It is just over ten years since Blakemore and Lord successfully produced an Eck's fistula in man (Blakemore & Lord, 1945). Good results following porta-caval anastomosis have been reported by Milnes Walker (1952, 1957) and Macpherson *et al.* (1956). Cavernomatous formation in the portal vein may make it difficult or impossible (Hunt, 1952), and lieno-renal anastomosis (Learmonth & Macpherson, 1948) offers an alternative procedure. This involves less adventurous surgery and lower mortality, but the anastomosis between tubes of a lesser bore is more likely to become occluded suddenly by a clot or progressively fibrosed, and so be effective for only a short time.

Any major shunt operation implies the formation of a large new portal-systemic channel and may be followed by the appearance of hepatic coma.

d. Coma

The importance of precipitating factors such as gastro-intestinal haemorrhage, paracentesis, operation and infection

[1] A further discussion of the treatment of ascites will be found in the paper by Sherlock, p. 139 of this number of the Bulletin.—Ed.

are stressed elsewhere in this Bulletin (see Sherlock, p. 136). Prophylaxis is important; a close watch must be kept on the protein content of the diet, and ammonium-containing medicines avoided.

The efficacy of treatment in established coma is difficult to evaluate. Fluid and electrolyte imbalance must be corrected and the caloric intake maintained. Glutamic acid, originally suggested to improve the ammonia-binding power of the brain (Walshe, 1953, 1955), has proved disappointing in the coma of acute hepatitis though it may be helpful in cirrhosis (Stokes, 1957). Attention is now focused on the role of intestinal intoxication and its control by antibiotics. Sherlock, Summerskill & Dawson (1956) have reported some success from using chlortetracycline, while Fisher & Faloon (1956) find neomycin of value[2].

6. Diet in Liver Disease

Considerable confusion still persists concerning the role of diet in the treatment of hepatic disorders: confusion made worse by unjustifiable deduction from animal experiment. Clinically it is clear that while the actual intake of carbohydrate and fat makes little difference to the prognosis in liver disease, that of protein is often extremely important. Just as in renal disease, progress of the disorder and the appearance of certain signs of failure may indicate the need for a sharp reduction of dietary nitrogen.

It is probable that in all patients with acute hepatitis the protein consumption should, when possible, be somewhat increased, while in patients with a lowered plasma albumin a high intake may prevent the onset of oedema and ascites and may help to relieve them if present. In chronic alcoholics marked improvement may follow the withdrawal of alcohol if a high-protein high-calorie diet is taken. In certain patients, however, especially those with portal hypertension and portal-systemic shunts, whether natural or surgically induced, rigid restriction of protein is indicated.

A further analogy can be drawn between hepatic and renal disease, for in both a low sodium intake may be of great importance. In the presence of ascites the sodium content of the diet should be about 200 mg. per day. The preparation of such a diet calls for considerable skill, and salt-free butter or margarine and bread are essential. If the protein intake is to be maintained despite sodium restriction, such preparations as sodium-free milk powder (Edosol) and calcium caseinate (Casilan) are invaluable.

It is important always to try to maintain an adequate calorie intake even if protein and salt are restricted, and in many cases it is wise to add vitamin supplements.

7. Conclusion

We have attempted to summarize the advances in treatment which have resulted during the last ten years from the improved understanding of liver disease. It is doubtful whether we shall see as much progress in the next decade. We may hope, however, for a clearer elucidation of the pathogenesis of hepatic coma and, if we are fortunate, chance will provide us with an antibiotic effective against the infective agent in hepatitis. Also, alert observation may tell us whether there are noxious substances in Britain analogous to the bush teas of the West Indies, which may be of importance in the aetiology of cirrhosis, and so open the way to prophylactic measures other than the control of acute hepatitis and the discouragement of chronic alcoholism.

[2] See also Sherlock, p. 138 of this number of the Bulletin.—Ed.

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HISTORICAL NOTES ON SOME EPIDEMIC DISEASES ASSOCIATED WITH JAUNDICE

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Exclusive of tropical yellow fever, the diseases which in epidemic form show jaundice as a common and often prominent feature are, Weil's disease (leptospirosis icterohaemorrhagica), infective hepatitis, and louse-borne relapsing fever.

Weil's Disease

This disease comes into a category different from the others that have been named, because, owing to its method of spread from rodents, the infection could not build up to a general epidemic involving large numbers of people over a wide area.

Nothing is known of the disease in early times, though no doubt outbreaks of it were included in the heterogeneous group that down through the centuries was labelled "jaundice"; and I have not myself encountered a description of likely cases of infection earlier than Marston's account, written in 1861, of two soldiers who, after bathing in muddy water in Malta, developed severe attacks of haemorrhagic jaundice, in one of them ending fatally (Marston, 1863).

This infection was differentiated from other forms of jaundice and established as a clinical entity by Weil of Heidelberg in 1886, hence "Weil's disease". Later it was closely studied in Japan where, in 1915, Inada and Ido isolated the causative organism now known as *Leptospira icterohaemorrhagiae*, although this leptospira is probably identical with Stimson's "(? *Spirochaeta*) *interrogans*" discovered by him in 1907 in a case of what was supposed to be yellow fever.

In severe infections the clinical resemblance between Weil's disease and yellow fever may be very close, as is shown by the strange story of Noguchi who by the weight of his authority unwittingly obstructed the study of yellow fever and led other investigators astray, having been himself misled in the first place by wrongful diagnoses. From persons diagnosed on clinical evidence as suffering from yellow fever in localities as far apart as Central America, Mexico, Peru, and Brazil, he had isolated an organism which he called "*Leptospira icteroides*", maintaining that it was distinct from the leptospira of Weil's disease. It is now clear that these local outbreaks were not yellow fever, as the clinicians thought, but were Weil's disease, and that the organism Noguchi had isolated over a period of years was, in fact, *Lepto. icterohaemorrhagiae*. In the end Noguchi's fruitless attempt to recover his "*Leptospira icteroides*" in cases of true yellow fever in tropical Africa, led to his tragic death in 1928 from an attack of this disease accidentally contracted.

Infective Hepatitis

In the older accounts of epidemics of wide-spread jaundice in temperate climates it is often impossible to distinguish between infective hepatitis and louse-borne relapsing fever, because of a lack of details sufficient for recognition. The confusion between the two is exemplified in the *World atlas of epidemic diseases* (Rodenwaldt, 1952) where a number of the epidemics cited as infective hepatitis were, in my opinion, relapsing fever; for they occurred at times of food shortage or actual famine when relapsing fever is known to have been rife (e.g. 1743, Ireland and Scotland; 1826, Dublin; 1827 and 1846, London—imported by fugitives from famine and fever in Ireland).

The case mortality, if stated or indicated, would serve to distinguish between the two. That of infective hepatitis is very low, only about 0.1%; whereas in relapsing fever the rate is much higher, and in different epidemics has varied from 6% to as high as 70%, according to their degree of virulence. Again, mention of severe rigors, profuse nose-bleeding, or relapses, would point to relapsing fever.

Extensive outbreaks of infective hepatitis are common in war, hence the old name, "camp jaundice"; for example, in the Federal Army in the American Civil War, 41,569 cases of jaundice were reported, with 161 deaths. However, the low case-mortality in "camp jaundice" does not reflect the military importance of a disease that has often caused serious, if temporary, wastage of man-power. The form of jaundice that attacked Napoleon's army in Egypt cannot have been this disease—as has been claimed—for Baron Larrey recorded that, among some 600 wounded soldiers, 260 died of it within a period of about seven weeks. The true identity of this epidemic remains debatable, though Larrey himself thought it to be yellow fever, a name then with a wider application than it has today.

In considering the nature of outbreaks of war jaundice in the past, it is well to remember that the epidemics of typhus, that old scourge of armies in the field, were often associated with relapsing fever in which the incidence of jaundice may be from about 20% to over 90% of cases, in general proportion to the intensity of the infection.

The earliest record of "epidemic jaundice" that Hirsch (1886) discovered in the course of his long researches was that given by Cleghorn, referring to an outbreak of jaundice in epidemic form in Minorca in 1745. In all, Hirsch collected details of 34 such epidemics, 15 of which were confined to bodies of troops. Some of the outbreaks affected small and defined circles of persons, others were somewhat more extensive, and still others were diffused over wide tracts of country. It is likely, however, that not every outbreak of jaundice in Hirsch's series was one of infective hepatitis; several of these where haemorrhages were a feature may well have been Weil's disease. He includes a most interesting and suggestive account of an epidemic of jaundice affecting the workmen in the shipyards of Bremen in 1883. Because of the occurrence of some cases of smallpox, extensive vaccinations were carried out. Of 1,289 men vaccinated with glycerinated lymph of human origin, 191 developed jaundice at intervals of from several weeks to two months. Whereas, among 500 of the men who were vaccinated at the same time but with different lymph, there was no case of jaundice; nor were there any cases in another group of 87 men who were vaccinated away from the shipyard, presumably with different lymph also. The observer who investigated the outbreak conjectured that

it was caused by " bad " lymph—probably true, though not in the way that he thought. Readers will remember that human serum was formerly used as the diluent in the preparation of yellow fever vaccine. Sometimes it happened that the donor of the serum was infected with a virus which set up a hepatitis with jaundice in a number of those vaccinated. I suggest as highly probable that some proportion of the human lymph used to vaccinate the first large group of the Bremen workmen had been taken from a virus-infected donor, and that the consequent jaundice was of the same nature as the outbreaks that proved so puzzling in vaccination against yellow fever many years later during the Second World War.

Louse-Borne Relapsing Fever

The causative organism of this infection was discovered by Obermeier in 1868; and in his honour Cohn named it *Spirochaeta obermeieri* in 1875. This name continued in use for many years and is still sometimes met with; but when it came to light that the organism had been given the specific name *recurrentis* by Lambert in 1874, by the Law of Priority the latter became the correct specific name. Since the species was first founded it has journeyed through a community of genera—*Protomycetum, Spirochaeta, Treponema, Borrelia*—and as it would be optimistic to hope that this pilgrimage is at an end, the second of these names—which no one can fail to recognize—is used for the purpose of these notes.

Descriptions of the disease, however, are to be found long before Obermeier's day, and there is an unmistakable one as far back as the Hippocratic *Epidemics*. This has often been taken as referring to malaria—because of the mention of rigors and relapses—by those not familiar with the disease. The most significant part of the description runs in W. H. S. Jones's translation:

> The great majority had a crisis on the sixth day, with an intermission of six days followed by a crisis on the fifth day after the relapse. Those who had a crisis on the seventh day had an intermission of seven days, with a crisis on the third day after the relapse . . . most had rigors near the first crisis, and those who had rigors at first near the crisis, had rigors again in the relapse at the time of the crisis.

The rigors occurred " by far the most during the winter ". The commonest complications of relapsing fever—jaundice and profuse epistaxis—are both mentioned.

After this impressive description there is no recognizable mention of relapsing fever for many centuries, although the disease must have persisted. It was long confused with typhus, and under the name " bilious typhus " was at one time viewed as no more than a clinical variant of that disease; one argument advanced to support this belief was that a patient's illness might begin as " spotted typhus ", and later assume the characters of " relapse fever "; or vice versa—obviously examples of double infection in diseases that so commonly occur together. I have expressed the belief (Mac Arthur, 1954) that the Greek epidemic, referred to above, also was accompanied by typhus, for there is described an acute fever with phrenitis, slight rigors, thirst, sleeplessness, much delirium, terrors, and depression; no severe epistaxis—if any, only slight; very fatal, but in favourable cases having a crisis from the eleventh to the twentieth day. There were no relapses. Included is the most suggestive statement, that although the comatose fever very commonly attacked children, of all patients these had the lowest mortality. The usual rarity of deaths in children, in contrast to the heavy mortality in adults, is a striking peculiarity of epidemic typhus. This Hippocratic account of the Thasos epidemics was clearly a patchwork compilation, for some of the parts show clinical acumen of a higher order than others. One interesting observation emerges as regards what was unmistakably relapsing fever, and what I believe was typhus. The physicians regarded epistaxis as a good sign in prognosis, and rightly so; but not, as they thought, because of any curative action, but because, I believe, the onset of profuse epistaxis in a patient indicated that relapsing fever, and not the more fatal typhus, was the infection present.

" Intermittent fever ", once a formal medical name for malaria, was in Ireland at one time applied to relapsing fever, and epidemics of the disease under this name are recorded for 1728 and 1729 in Dublin, being " accompanied by some of the petechial kind [typhus] ". The brief note given by Rutty (1770), the Quaker physician of Dublin, is often wrongly claimed to be the first clinical description of the disease; so it may be quoted for comparison with that of the Greeks which pre-dated it by more than 2,000 years:

> It seems not unworthy of notice that through the three summer months [June, July and August, 1741] there was frequently here and there a fever altogether without the malignity attending the former [typhus], of six or seven days duration, terminating in a critical sweat, as did the other also frequently, but in this the patients were subject to a relapse, even to a third or fourth time, and yet recovered.

It is sufficient to mention that epidemics of relapsing fever were a regular part of the recurring famines of the 18th and 19th centuries in Ireland, for fever has always stalked at the heels of famine. In these conditions the disease was observed to spread much more rapidly than the associated typhus, and to run through whole families in a few days—a strange difference, considering that both infections are louse-borne. I believe that the rapid dissemination of relapsing fever was due to the common and profuse bleeding from the nose—often intensified by coexisting scurvy—which soaked the sufferer's bedding and surroundings, so that the other occupants of the crowded cabins could not escape contamination with blood potentially infective through the skin or the eye. It may be added that infection has often taken place among the staff of hospitals and laboratories where the precaution of wearing rubber gloves, when handling specimens of blood taken from relapsing-fever patients, was neglected.

Louse-borne relapsing fever of great virulence broke out in North Equatorial Africa in 1921, and swept across the continent causing hundreds of thousands of deaths. For example, in the French Sudan and the Niger about 100,000 persons perished, and in the upper Volta area, 20,000; spreading from British and French West Africa, it attacked the Anglo-Egyptian Sudan, and here at least 200,000 deaths resulted. In untreated cases, the mortality was between 60% and 70%. The incidence of jaundice varied in different localities from 50% to 100% of those attacked. The virulence of the infection, the intense jaundice sometimes with black vomit, and the high death-rate, at first suggested yellow fever; but *Spirochaeta recurrentis* was proved to be the organism responsible.

Normally the mortality from relapsing fever is low as compared with that of, say, typhus or smallpox; but this calamitous African epidemic exemplifies the degree of severity that the disease may reach, even today, if the virulence and

diffusive power of *Spirochaeta recurrentis* become enhanced by some agency of uncertain nature; how much more readily in ancient times might a disastrous wave of pestilence have swelled up, through some strange conjunction of circumstances. And this reflection is a fitting prelude to the section that follows.

The Yellow Plague

The depopulation and wide-spread dearth resulting from the pandemic of bubonic plague[1] with which the Emperor Justinian's name has been joined, prepared the way for the pestilence that followed close on its heels. This was called the Pestis Flava in Latin, the Buidhe Chonaill and the Crón Chonaill by the Irish annalists,[2] and the Fad Felen in Welsh—all with the meaning, the Yellow Plague. The date assigned to it in the Irish annals varies by several years around A.D. 550—the entry for 555 in the Annals of Ulster is a copyist's error (Mac Arthur, 1949). There is a mistaken idea that, because some of the early dates in the annals are manifestly wrong, the related entries are therefore unreliable. But the A.D. dates were inserted by annotators centuries afterwards, when the complicated Celtic system—84-year cycles with distinguishing lunar and ferial data—was replaced by the A.D. chronology, and many mistakes were made in the difficult calculations involved, some of which have been corrected by scholars of today.

It is likely that the Justinian Plague and the Yellow Plague may have overlapped to some extent, but the Irish annalists differentiate the two by their distinctive names, and state further that the former was the "first Mortality," as if to demarcate it from the second that followed. In some late recensions of older texts—e.g. the Book of Landaff, a British chronicle—the two epidemics are confused and run together as one, under the name the Yellow Plague; and I believe that the fanciful account of its horrors, given in the text mentioned, was inspired by some memory of the more terrible Justinian Plague, which caused a mortality no less heavy than that of the Black Death eight centuries later.

The unanimity both in Britain and Ireland in applying the descriptive term "yellow" to this pestilence, shows that there must have been some such discoloration of the skin. Shrewsbury (1949) propounded the theory that the epidemic was confluent smallpox of great severity, the running together of the pocks masking the nature of the disease. To me this is incredible. I have seen confluent smallpox of every degree of severity, and even when the whole face was one superficial abscess, the pocks on the trunk remained discrete and easily recognizable for what they were. No cases could be more severe than those I refer to, and yet be confluent; because if they were still more virulent, the subjects would have died before the rash developed, or before it had time to become fully confluent. Besides, no epidemic could remain wholly confluent throughout; and as its initial fury was exhausted, discrete and unmistakable cases of the disease would become correspondingly numerous. The ancient Irish were familiar with smallpox and called it by descriptive names of their own; and had the epidemic in question been that disease, they would have said so plainly, as they did of other epidemics of smallpox in the same century. In Ireland the term "yellow" was never used to describe smallpox at any period.

I believe that the characteristic yellowness was caused by jaundice; and the only infection that could give rise to a fatal epidemic general over Britain and Ireland, with jaundice as a striking feature, is relapsing fever. I first thought of this identification years ago when listening to an aged narrator who was recounting events of the Irish famine of 1846–50; having talked of "the spotted fever" [typhus], he went on to describe "the yellow fever" [relapsing fever], and explained that it was given this name because "those who caught it were coloured yellow with the jaundice." At once there recurred to my mind an identical explanation in the Book of Landaff referring to the epidemic of fourteen centuries before: "It was called the Yellow Plague because it occasioned all persons who were seized by it to be yellow." And the further evidence collected over the intervening years leaves no doubt in my mind regarding its true identity. Thus, I may mention that relapsing fever was long called "the yellow fever", not only in common usage but by famous figures in medicine as well—e.g. Lind, and a century later, Stokes, and Graves; in the last-named author's text-book, the chapter on relapsing fever is headed: "Yellow Fever of the British Islands." And perhaps this name might be heard even today in a fireside story of the last great famine in Ireland, just as it is to be found in contemporary medical reports of that terrible time. Nor was the name peculiar to these islands: the "Yellow Disease" of the Shantung districts of China, investigated some 30 years ago, was found to be relapsing fever of the louse-borne kind.

In my opinion there can be no doubt that the Yellow Plague of the sixth century was relapsing fever of a severe type, in which jaundice was common enough to dominate the picture of the disease in the popular mind.

The False "Yellow Plague" of A.D. 664

The account that follows is largely drawn from a discussion of the subject elsewhere (Mac Arthur, 1949).

In some transcripts of the Irish annals the name Buidhe Chonaill (Yellow Plague) unfortunately became attached to the great epidemic of 664 and, originating solely from this source, the identification has been adopted in editorial notes in several printed translations of the Venerable Bede's writings and in other works; and thence has spread far and wide. At one time I accepted it as probably a genuine and a contemporary name, though a most perplexing one because, being familiar with bubonic plague, I could conceive of no possible reason for calling an epidemic "yellow" which I did not doubt was this disease.

Bede's story of this epidemic is well known. In his Ecclesiastical History he tells of how a sudden pestilence depopulated the southern parts of Britain, and then extending northwards, ravaged the country far and near and destroyed a great multitude of men. He adds that the pestilence did no less harm in Ireland, where many of the English nation were then pursuing their studies or leading monastic lives. From other sources we know that it also reached Scotland. Neither in Bede's own Latin nor in the version in Anglo-Saxon is the pestilence given any descriptive name; but the account as a whole is strongly suggestive of bubonic plague, and might

[1] To this diagnosis, Zinsser (*Rats, lice and history* (1935), p. 147) made the startling addition "... references to the general eruption of black blisters in many cases indicate that smallpox of a very severe type participated." These "black blisters" were, of course, plague blains.

[2] *Buidhe*, pronounced "bwee", means yellow; and *crón*, yellow-brown. It is noteworthy in this connexion that the relapsing-fever jaundice of 1846 varied "from a pale sallow unhealthy colour, to the deepest jaundice of a dusky orange brown."

serve equally well as a summary of the course of the Black Death in this country.

In early times the whole concept of bubonic plague was centred on the agonizing inflammation of the lymph-glands, especially those in the most common site—the groin and adjoining thigh—where there may be a swelling as large as a hen's egg or one's fist. For this reason the ancient Greeks called the disease " pestilential groins ", whence our word, bubo; and the Romans, the " groin plague " (*pestis inguinaria*). But in another work of Bede's, his Life of St Cuthbert, he introduces a most significant diagnostic detail in quoting the Saint's own story of " the tumour that arose in his thigh " when he himself (Cuthbert) " was stricken down with the pestilence which at that time carried off so many throughout the length and breadth of Britain." The context and the matter-of-course way in which Bede associates the Saint's tumour with the pestilence then raging, imply that a " tumour" (bubo) in that site was the accepted feature of an attack. And plague is the only killing epidemic disease which is characterized by buboes in the groin and thigh.

How, then, did the mistaken identification come about? First, it must be explained that when the manuscripts of the annals became worn by handling, new transcriptions were prepared; and in these a copyist on occasion introduced into the body of the text various notes or comments that some person had written in the margin of the page he was copying. I first began to doubt the authenticity of the name Buidhe Chonaill for the plague of 664, on observing that where it appears in the text of a chronicle it is in a form that suggests a copyist's interpolation—that is, it breaks into the smooth flow of the wording. As one example: " Of that mortality, i.e., of the Buidhe Chonaill, these two kings of Ireland died." (*Marbh tra don mortlaid sin .i . do'n Buidhe Conaill, na dá righ si Eirenn.*)

My suspicion that Buidhe Chonaill was neither a genuine nor a contemporary name for this plague was confirmed by the fact that it is not given in the Annals of Inisfallen, the oldest of all the surviving manuscripts and the one most free from interpolations. There are two manuscripts of the Annals of Ulster, one preserved in Trinity College, Dublin, the other in the Bodleian Library, Oxford; and I have examined both. In the Trinity copy, " Buidhe Chonaill " has been written in between the lines in a hand and ink different from those of the relative text, and is an addition by a later scribe. In the Bodleian copy, the name does not occur at all in this context. It is evident, therefore, that it was not in the earlier manuscript from which both of these copies were transcribed, nor in its pre-existing prototype compiled, it is believed, about A.D. 740 in the abbey of Bangor (County Down) from yet more ancient manuscripts, which embraced the period of the plague of 664, for we still know the names of the four abbots of the Bangor abbey who perished in the pestilence; and had it been called the Buidhe Chonaill by those who passed through it, this name would have been handed down in the same way.

It is my reasoned conviction that the great epidemic of 664 was bubonic plague, and that in Ireland it was never supposed to be a second visitation of the Yellow Plague until some meddling scribe, writing at a date several centuries after the event, inserted the name as a retrospective gloss in the mistaken assumption that this pestilence had been of the same nature as the true Buidhe Chonaill of the century before.

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Notes on Contributors

DR W. H. HORNER ANDREWS is Lecturer in Pharmacology, University of London, at St Mary's Hospital Medical School. From 1943 to 1946 he served in the Royal Army Medical Corps in India, being first Pathologist, and later, Specialist Physiologist. In 1946, he gained the Diploma in Tropical Medicine and Hygiene, Liverpool, and he was Lecturer in Tropical Medicine at the University of Liverpool, 1946–54, where he collaborated with Professor B. G. Maegraith. Dr Andrews has worked mainly on the vascular system of the liver, with special reference to the vascular element in the pathogenesis of liver damage, and also on the treatment of malaria. At the present time he is working chiefly on the factors involved in biliary secretion, and the relation of hepatic function to blood vessels and blood flow.

DR G. BRAS worked for many years in Indonesia; he was a prisoner of war to the Japanese, 1942–45. He is a morbid anatomist and was Professor of Pathology in the University of Indonesia, subsequently Senior Lecturer and Reader in Morbid Anatomy, University College of the West Indies, where he was recently appointed Professor of Pathology. While in Jamaica, the histopathology of the malnourished child, and the morphology and aetiology of juvenile cirrhosis of the liver, have been the main objects of his studies.

DR C. B. CAMERON is Lecturer in Chemical Pathology at St George's Hospital Medical School, London. He graduated in medicine from the University of Otago, New Zealand, in 1945, and two years later came to London where, since 1951, he has worked on various clinical and biochemical aspects of steroid hormone metabolism. Publications on urinary steroid excretion in liver disease have appeared in the *Journal of Endocrinology*.

DR EDWARD R. CULLINAN is Physician to St Bartholomew's Hospital, the Westminster (Gordon) Hospital, the King Edward VII Hospital for Officers, and Honorary Consulting Physician to the Army and to the Royal Hospital, Chelsea. He was formerly honorary editor of the *Proceedings of the Royal Society of Medicine*. In 1950 he was awarded the Bradshaw Lectureship of the Royal College of Physicians, and in 1953 he gave the Lettsomian Lectures to the Medical Society of London. During the war he served with the Royal Army Medical Corps in the Middle East and later was Brigadier Consulting Physician to the East Africa Command in 1944 and 1945. He is a general physician whose special interest is the study of diseases of the liver and abdomen, and he has published many articles on this subject.

PROFESSOR J. N. DAVIDSON was appointed to the Gardiner Chair of Physiological Chemistry in the University of Glasgow in 1948. He contributed a paper entitled "Enzymes acting on nucleic acids" to an earlier number of the Bulletin (*Brit. med. Bull.* 1953, **9**, 154), with which a biographical note was published. Recently, in collaboration with Dr E. Chargaff, he has edited a comprehensive treatise, *The nucleic acids: chemistry and biology* (1955); and the 3rd edition of *Textbook of physiology and biochemistry*, of which, together with G. H. Bell & H. Scarborough, he is author, is reviewed on p. 152 of this number of the Bulletin.

PROFESSOR C. H. GRAY is Professor of Chemical Pathology in the University of London, Chemical Pathologist at King's College Hospital, London, and Honorary Lecturer in Biochemistry at University College, London. The work of his Department at King's College Hospital is concerned with hormone disturbances in diabetes and pregnancy and the effect of salicylates on carbohydrate metabolism, in addition to work on the porphyrins and bile pigments. Professor Gray contributed a paper on isotope studies in porphyria to an earlier number of the Bulletin (*Brit. med. Bull.* 1952, **8**, 229).

DR R. D. HARKNESS is a Reader in Physiology at University College, London. He received his medical and scientific education at University College and Hospital and graduated in 1942. After the war he returned in 1946 to University College as Lecturer in Physiology. In 1952 he spent a year at the National Institute for Medical Research with Professor A. Neuberger. He is interested primarily in problems of growth and morphogenesis. At first he worked on regeneration of liver and became particularly interested in the behaviour of the connective tissue elements. This has led, more recently, to a more general interest in the connective tissues of the other parts of the body, especially in the reproductive tract. He has published papers in the *Journal of Physiology*, *Biochemical Journal*, *Proceedings of the Royal Society* and the *Quarterly Journal of Experimental Physiology*.

SIR HAROLD HIMSWORTH was appointed Secretary to the Medical Research Council in 1949. He had been Professor of Medicine, University of London, and Director of the Medical Unit, University College Hospital, London, from 1939 to 1949. In 1939, he gave the Goulstonian Lectures; in 1949, the Oliver-Sharpey Lectures, Royal College of Physicians, and the Sydney Ringer Lectures; and in 1947 he delivered the Lowell Lectures at Boston, Massachusetts, which he later published as *Lectures on the liver and its diseases* (1947; 2nd ed., 1950). He is Honorary Member of the Medical Society of Sweden, Corresponding Member of the American Association of Physicians, Hon. D.Sc. (Manchester), LL.D. (London), LL.D. (*hon. caus.*), (Glasgow), and Docteur (*hon. caus.*), University of Toulouse. In 1956 he also became Honorary Member of the Belgian Royal Academy of Medicine.

DR J. C. HOUSTON is an assistant physician and Sub-Dean of the Medical School at Guy's Hospital, London. During the Second World War he served with the Royal Army Medical Corps in India Command as a medical specialist, with the rank of major, and on demobilization he was appointed Medical Registrar and subsequently Clinical Tutor at Guy's. In 1950 he spent three months in the United States of America on a travelling Fellowship and made a similar tour of European medical centres in 1953. His published work includes: "Phlebotomy for haemochromatosis" (*Lancet*, 1953, **1**, 766); "Iron metabolism" (*Post Grad. med. J.* 1955, **31**, 394); with T. M. L. Price, "Addison's disease" (*Guy's Hosp. Rep.* 1948, **97**, 254); with R. H. S. Thompson, "The diagnostic value of serum iron studies in haemochromatosis" (*Quart. J. Med.* 1952, n.s. **21**, 215); and with S. De Navasquez & J. R. Trounce, "A clinical and pathological study of fatal cases of status asthmaticus" (*Thorax*, 1953, **8**, 207). He is also Assistant Editor (to Sir Russell Brock) of the *Guy's Hospital Reports*.

SIR WILLIAM MAC ARTHUR is Advisory Editor, *British Medical Bulletin*, and Editor, *Transactions of the Royal Society of Tropical Medicine and Hygiene;* University Lecturer in Tropical Medicine, Oxford, and Consultant, Royal Masonic Hospital, London. He was Professor of Tropical Medicine and later Commandant and Director of Studies, Royal Army Medical College. Among other activities he has carried out researches on medical problems in the early Celtic annals; and contributed the medical section to *The great famine: studies in Irish history, 1845–52* (1956). He has been Vicary, Cavendish, and Honyman Gillespie, Lecturer on medico-historical subjects; and contributed a paper on the history of English malaria to *British Medical Bulletin* (1951, **8**, 76).

PROFESSOR N. F. MACLAGAN has been Professor of Chemical Pathology at Westminster Medical School London, since 1947. He was Assistant in the Courtauld Institute of Biochemistry from 1926 to 1935; and biochemist at the Westminster Hospital from 1935 onwards. During the period 1944–50, his work was mainly on liver function, but his present work is chiefly on thyroid function and mucoprotein metabolism. His publications include: "The thymol turbidity test as an indicator of liver dysfunction" (*Brit. J. exp. Path.* 1944, **25**, 234); with J. H. Wilkinson, "The thyroid hormone" (*Rep. Progr. Chem.* 1952, **49**, 291); and, with E. Lockey & A. J. Anderson, "Urine and serum mucoproteins in cancer and in other diseases" (*Brit. J. Cancer*, 1956, **10**, 209). Professor Maclagan was Honorary Secretary to the Society for Endocrinology from 1951 to 1954; Honorary Secretary to the Board of Studies in Biochemistry (University of London) from 1951 to 1953, and President of the Association of Clinical Biochemists from 1954 to 1956.

DR P. N. MAGEE studied medicine at the University of Cambridge and University College Hospital, London. After the usual junior appointments and service with the Royal Air Force Medical Branch he joined the Graham Research Department of University College Hospital Medical School under Professor G. R. Cameron, F.R.S., first with a Medical Research Council grant; later he held the Graham Scholarship in Pathology. He is now a member of the Scientific Staff of the Medical Research Council's Toxicology Unit, Carshalton. His published work includes papers on anhydraemia, acute inflammation, and the pathology and biochemistry of various toxic conditions. At present he is studying the mechanism of action of poisons, with special reference to toxic liver injury.

PROFESSOR N. H. MARTIN is on the staff of St George's Hospital, London, and holds a Chair of Chemical Pathology in the University of London. After graduating with honours in chemistry he continued postgraduate work in Germany and at the Imperial College, London. Subsequently graduating in medicine at Oxford University and Middlesex Hospital, London, his interest in liver damage was first stimulated when working under Dr E. A. Cockayne in 1937. During the 1939–45 war, he made an intensive study of hepatitis in Africa with Dr G. M. Findlay, and continued his studies of the problem of protein metabolism in relation to liver damage, in 1946 and 1947 at Harvard University, USA. He is at present extending his studies of the intimate changes of protein structure in established disease processes.

PROFESSOR A. NEUBERGER has since 1955 been Professor of Chemical Pathology at St Mary's Hospital Medical School, London. Prior to this he was for ten years a member of the Scientific Staff of the Medical Research Council at the National Institute for Medical Research, London. Professor Neuberger continues to pursue his studies on porphyrins, proteins and amino-acid metabolism. He was Chairman of the Editorial Board of the *Biochemical Journal* between 1952 and 1955. He has contributed two papers previously to the Bulletin: one in 1948 on amino acids in nutrition (*Brit. med. Bull.* 1948, **5**, 346), and a second in 1952 on protein metabolism (*Brit. med. Bull.* 1952, **8**, 210). Professor Neuberger was elected a Fellow of the Royal Society in 1951.

PROFESSOR M. L. ROSENHEIM has been Professor of Medicine in the University of London and Director of the Medical Unit, University College Hospital Medical School, since 1950. He was educated at Cambridge and at University College Hospital, and,

in 1935, introduced mandelic acid in the treatment of urinary infection. After holding the appointment of Medical Registrar at University College Hospital, he went to the United States of America as Bilton Pollard Travelling Fellow and worked as a research assistant at the Massachusetts General Hospital. He served in the army from 1941 to 1946, being Consulting Physician to the Allied Land Forces, South-East Asia, 1945–46. On return from the army he was, for some time, Secretary of the Food Rationing (Special Diets) Advisory Committee of the Medical Research Council. Since the war he has been mainly interested in problems concerning renal disease, hypertension and endocrinology. Professor Rosenheim's publications include: "Mandelic acid in the treatment of urinary infections" (*Lancet*, 1935, **1**, 1032); "The treatment of severe hypertension" (Oliver-Sharpey Lectures at the Royal College of Physicians, 1954) (*Brit. med. J.* 1954, **2**, 1181); and, with P. Arnold, "Effect of pentamethonium iodide on normal and hypertensive persons" (*Lancet*, 1949, **2**, 321).

DR SHEILA SHERLOCK is Lecturer in Medicine, Postgraduate Medical School of London, and Physician to Hammersmith Hospital, London. Earlier, she was Clinical Tutor, Royal Infirmary, Edinburgh; Beit Memorial Research Fellow, Postgraduate Medical School of London; and Rockefeller Research Fellow at Yale University, USA. She has worked on carbohydrate metabolism, on diabetes and various aspects of liver disease; her current work is on the mechanism of hepatic coma and on portal hypertension. Among Dr Sherlock's publications are: *Diseases of the liver and biliary system* (1955); with J. H. Dible & J. McMichael, "Pathology of acute hepatitis" (*Lancet*, 1943, **2**, 402); and, with W. H. J. Summerskill, E. A. Davidson & R. E. Steiner, "The neuropsychiatric syndrome associated with hepatic cirrhosis and an extensive portal collateral circulation" (*Quart. J. Med.* 1956, n.s. **25**, 245). Dr Sherlock has recently made lecture tours to South African universities, to the British University in the West Indies, and to universities in Canada and the USA.

DR J. F. STOKES trained at Cambridge and University College Hospital, London, qualifying in 1937. He joined the Royal Army Medical Corps in 1942 and spent the greater part of his war service with the 14th Army in Assam and Burma. He found much material there with which to indulge his interest in liver disease, and his first papers were based on the observation of the particularly severe form of hepatitis which occurred in the Far East. On his return to England he was appointed Assistant Physician to University College Hospital, was awarded "proxime accessit" for the Raymond Horton-Smith prize for his M.D. thesis, and became Thruston medallist of Gonville and Caius College, Cambridge. He now practises as a general physician while retaining a special interest in liver disease. Dr Stokes recently reviewed the experience at University College Hospital over the last decade in the treatment of hepatic coma (*Lectures on the scientific basis of medicine* (1957)).

DR H. B. STONER has previously contributed an article to the Bulletin with Professor H. N. Green, entitled "Effects of injury on carbohydrate metabolism and energy transformation" (*Brit. med. Bull.* 1954, **10**, 38), with which a biographical note was published. He is a member of the Medical Research Council's Toxicology Research Unit at Carshalton, Surrey, and is concerned with the reaction of the body to physical and chemical injury.

DR DAVID SUTTON is at present the Assistant Director of the Radiological Department at St Mary's Hospital, London, and Consultant Radiologist at the Maida Vale Hospital for Nervous Diseases, London. He graduated in medicine at Manchester University Medical School in 1942, and after army service held radiological appointments at the Royal Free Hospital, The National Hospital, Queen Square, and the Middlesex Hospital, London. His chief radiological interests are in radiology of the nervous system and vascular radiology. His publications include a chapter on neuroradiology in the 6th edition of *Recent advances in neurology and neuropsychiatry* (1955); a chapter on arteriography in the forthcoming edition of *A textbook of x-ray diagnosis by British authors*; and, with J. W. D. Bull, "The diagnosis of paraphysial cysts" (*Brain*, 1949, **72**, 487). Dr Sutton gained the Röntgen Award of the British Institute of Radiology in 1952.

DR J. M. WALSHE is Assistant in the Medical Unit, University College Hospital Medical School, London. During 1954–55, he was Bilton Pollard Travelling Fellow of that Medical School, visiting the United States of America. Whilst there, he held the post of Research Fellow in Medicine at Harvard Medical School, working on liver disease with Dr Charles Davidson in the Thorndike Memorial Laboratory of the Boston City Hospital. Three of his recent publications are: "Penicillamine, a new oral therapy for Wilson's disease" (*Amer. J. Med.* 1956, **21**, 487); "Hepatic coma" (*Post Grad. med. J.* 1956, **32**, 467); and, with F. Challenger, "Foetor hepaticus" (*Lancet*, 1955, **1**, 1239). Dr Walshe has contributed a paper, together with C. E. Dent, on amino-acid metabolism to an earlier number of the Bulletin (*Brit. med. Bull.* 1954, **10**, 247).

DR J. C. WATERLOW was employed during the war by the Medical Research Council in investigations on methods of increasing the efficiency of military personnel in hot climates. In 1943, with Dr W. S. S. Ladell, he was sent to Iraq to study the problem of heat-stroke and heat-exhaustion. He later joined the staff of the Medical Research Council's Human Nutrition Research Unit, under Professor B. S. Platt, and worked in the West Indies, Basutoland, and in the Gambia, on malnutrition and liver disease in the tropics. In 1954 he was appointed Director of the Tropical Metabolism Research Unit of the Medical Research Council at the University College of the West Indies, Jamaica. His interest is in the common ground between physiology and medicine. Dr Waterlow contributed a paper on heat-stroke and heat-exhaustion in Iraq to an earlier number of the Bulletin (*Brit. med. Bull.* 1947, **5**, 3).

Book Reviews

Diseases of the Liver and Biliary System

Sheila Sherlock. Oxford: Blackwell Scientific Publications, 1955 (reprinted 1956). xv + 720 pages; 198 figures. 23 × 15 cm. £2 10s.

Diseases of the Liver

Edited by *Leon Schiff.* London: Pitman Medical Publishing Co. Ltd., 1956. xix + 738 pages; 244 figures. 26 × 19 cm. £5 10s.

These two books are in sufficient contrast to provide no conflict in the mind of the prospective buyer. Dr Sherlock's account of diseases of the liver is a confident black-and-white picture of what she sees and how, in her view, it happens. It is a workmanlike exposition and has the authority which rightly stems from her outstanding contributions in this field. The chapters on gall-bladder disease are not so convincing, and the author's less intense interest in this section of the book is apparent to the reader. Emphasis is laid on the experimental and laboratory aspects of liver disease, both human and animal, and Dr Sherlock's main objective is to rationalize the diagnosis and treatment of liver disease as far as possible at the present time. This is a laudable intent, for the most part realized, and her book can be wholeheartedly recommended to the thoughtful doctor who has patients with liver disease under his care. He should remember that he is reading a personal expression of experience, which may be amplified or denied by that of others, and that words such as "inexplicable" (p. 39) should be read as "unexplained".

The text would have been enlivened by brief case histories but lack of space probably forbade this. Fifty-seven of the 705 pages are devoted to the biochemistry of liver disease, even in the face of the statement (p. 67) that "The place of biochemistry in the practical diagnosis of liver disease has been over-emphasised", but this section could hardly have been compressed. The colour plate illustrating "liver palms" (fig. 33) would have been more telling if a greater part of the forearm had been shown in contrast. Alcohol is prohibited for six months after acute hepatocellular failure on p. 141 and for twelve months on p. 270. Some of the *x*-ray illustrations of gall-bladder disease have not come out well. These minor defects are outweighed by a good chapter on the haematology of liver disease, a useful list of foods containing large, medium and small amounts of salt, and by the author's clear exposition of the concept of portal-systemic encephalopathy.

Professor Schiff's book, in the production of which he has elicited the help of 27 contributors, is over half as long again. Greyer in tone, much of it demands more of the reader who is used to the predigestive assistance of Dr Sherlock's gastric juices; it is more comprehensive but less conclusive. We have seen the greater part of this team in informal action in the Macy Foundation Reports on Liver Injury, but the hope that a coherent and flowing account of liver disease might result from this present joint effort has not been wholly realized. This is a book for the specialist and research worker in the hepatic field. Nearly every chapter will stand on its own and inevitably there is overlap of subject-matter, divergence of views and unevenness of treatment. These features do not deny the real value of the book as a collection of extended essays written

from particular view-points. Any reader interested in toxic hepatitis, for instance, will find 441 references at the end of a 48-page article on the subject. Biochemical consideration is given full weight, though the diagrams are of variable quality; the central role of acetyl coenzyme A in intermediary metabolism is lucidly displayed (p. 75), but it needs an elementary training in model railway lay-outs as well as basic biochemical knowledge to appreciate the citric acid cycle as portrayed on p. 70.

Tumours of the liver are dealt with from the pathological standpoint without reference to C. Berman's useful monograph, *Primary carcinoma of the liver* (1951) and without an adequate account of the clinical aspects of secondary hepatic metastases. The chapter with the title "Diseases of the gallbladder and extrahepatic bile ducts" would be better entitled "The surgery of the biliary tract". Due credit is given to C. H. Gray's researches on bile pigments and to the British work on needle biopsy, but no mention is made of Milnes Walker's contribution to the surgery of portal hypertension, and there is an implication of splendid disregard of the Guy's Hospital tradition, in the term "addinsonian pernicious anaemia" (p. 444). J. R. Neefe has written an eminently sensible chapter on viral hepatitis, and among Professor Schiff's own contributions are three useful pages on pitfalls in the differential diagnosis of jaundice.

Britain contributes a convincing account of liver function tests by N. F. Maclagan and a beautifully concise essay on hepatolenticular degeneration by W. B. Matthews, while Dr Sherlock has rearranged sections of her own book to provide a chapter on the liver in circulatory failure.

The bibliography is full and the book will certainly be found on the shelves of most libraries but, apart from any other considerations, its higher cost will ensure a wider sale of Dr Sherlock's volume to the individual buyer.

J. F. Stokes

Textbook of Human Anatomy

J. D. Boyd, W. E. Le Gros Clark, W. J. Hamilton, J. M. Yoffey, S. Zuckerman, and the late A. B. Appleton. Edited by *W. J. Hamilton.* xii + 1022 pages; 797 figures. London: Macmillan & Co. Ltd., 1956. £5

The problem of structure and its development, and of the changes which it undergoes in different functional states, is perhaps the most fundamental of all biological problems. It is this problem, or complex of problems, which forms the subject-matter of the science of anatomy, and it is unfortunate that in the text-books most commonly used by medical students anatomy has come to be conceived in much narrower terms. Many of these books are admirable as detailed and accurate accounts of all those features of the adult human body which, with the aid of apparatus little more complex than the forceps and scalpel, can be observed with the naked eye. Microscopical and developmental aspects of structure or functional interpretation are not entirely ignored, but they occupy relatively a small part of these very large books. It is not surprising therefore that the student, early in his medical career, is imbued with the idea that anatomy is no more than an encyclopaedic knowledge of the "facts" of gross bodily structure, many of which have little intrinsic interest.

No doubt what has been said overstates the case, but it does represent a real situation and an attitude of mind which is widespread among medical graduates, whether their primary interests lie in the clinical field or in the basic medical sciences. For this reason, the appearance of a new text-book, designed to break with the tradition that anatomy is primarily a detailed descriptive account of structure mainly at the naked-eye level, is an important and welcome event. It is the more important because, in their research interests, anatomists themselves have for many years broken with this tradition.

The main objects of this new text-book are stated clearly in the preface: to establish a scale of values in relation to topographical detail, with the inevitable omission of much which appears in other text-books; to establish a closer relation between the study of structure and function; and to treat anatomy as " . . . an independent branch of biological science with problems and achievements of its own". A great advance has undoubtedly been made towards achieving these objects. As in most text-books of anatomy, the systems of the body are described separately, but each is introduced by a general account in which microscopical and functional aspects are set in their proper relationship to topographical description, giving it both interest and significance. The muscles, bones and joints are taken together as a "locomotor system" and, though this may cause a little difficulty for the elementary student when he first approaches the subject, it is a very real advantage in later stages when synthesis becomes essential for functional interpretation. The ductless glands are dealt with very fully, as their importance justifies, and "growth" is the subject of a most useful chapter in which some account is given of the methods by which it may be measured and recorded. The abdominal viscera are described clearly on fairly orthodox lines, and are well illustrated. The account of the liver, of particular interest as the main subject of this number of the Bulletin, is a good example of a short but clear description in which both gross and microscopical structure are taken into consideration, but it is a little disappointing to find no discussion of modern views on the liver lobule.

It is of course easy to criticize any work which deals with an old and established subject in a new way; every omission will be regretted by someone, and a number of errors or inaccuracies inevitably creep into a first edition dealing with so wide a field. Some can easily be found, but they are not important and can be corrected in a future edition which, one hopes, will soon be needed. The book is a valuable contribution to anatomy, and will help greatly to re-orientate the study of human structure towards its integration with the study of function and growth and their disorders. Perhaps the most valid criticism is that it does not go far enough in the direction it has taken. One would like to have seen more emphasis placed on methods of investigation and on the difficulties and often controversial nature of the interpretation of evidence. As soon as one goes beyond the simpler features of structure which can be observed with the naked eye, most of the so-called "facts" are matters of interpretation, and it is often more illuminating to know how the observations have been made and what the evidence is for a particular interpretation, than simply to record the facts so that they can be memorized. References to original literature would have been helpful, but these are almost entirely lacking. A most important step forward has been made, however, and both authors and publishers are to be congratulated on the production of a text-book which is far more readable and far closer to modern ideas and requirements than any we have had before.

F. Goldby

Textbook of Physiology and Biochemistry

George H. Bell, J. Norman Davidson & Harold Scarborough. 3rd ed. Edinburgh & London: E. & S. Livingstone Ltd., 1955. xii + 1068 pages; illustrations. 25 × 17 cm. £3

The appearance of a third edition of this well-known work is a sufficient indication of its popularity. The book aims at giving a general account of physiology and biochemistry roughly to the level of the second examination for medical degrees, but is unusual in the degree of integration which has been achieved between the two subjects. This is greatly to the advantage of the medical student who can find not only an adequate account of both subjects, but will gain an unusual insight into the influence of one upon the other. Students reading for Honours Degrees in physiology and biochemistry can also use the book with great advantage during their first one or two years.

The arrangement of the book follows that of previous editions, and the approach is through biochemistry to a study of function. The illustrations are all first class and well labelled, and include a considerable number of radiographs. In spite of the title, the authors have not hesitated to introduce clinical and pathological material where this is appropriate, particularly in the sections on the endocrine glands.

It is somewhat of an achievement to have compressed so much material into such a readable account of only 1,000 pages, and the book can be strongly recommended to students preparing for the examinations indicated, and also to clinicians wishing to refresh their knowledge of the basic medical sciences.

N. F. Maclagan

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Autonomic Nervous System: Introduction

When the Advisory Editor of the *British Medical Bulletin* honoured me with an invitation to write some kind of introduction to this number, I looked forward with unusual interest to the opportunity of reading the articles in which various authors, each of them from his own special angle, were presumably to deal with more or less recent advances in knowledge of the Autonomic Nervous System. I became conscious at once, however, of an inclination to wonder how many discoveries these relatively recent decades might have yielded, which could be regarded as concerned directly, or in any strict sense, with the structure and the functions of this particular section of the nervous system. I have also been conscious, of course, of what is doubtless in smaller degrees a matter of more general experience, in finding myself now increasingly unable to maintain more than a glancing contact with the records of what has been happening, even in the special field of the activities and interests which once were mine in a fuller sense. Making all proper allowance, however, for personal inadequacy of this kind, I still found it difficult to convince myself that it would be possible to muster, for discussion, a range of such relatively new advances in knowledge of the autonomic system itself, sufficient to supply the material for a Bulletin of

the dimensions which have come to be regarded as appropriate to this series. I found myself inclined, accordingly, to expect that some, at least, of the prospective contributions would be found to deal with fields of physiological investigation, at present rather widely and intensively cultivated, into which the first entry was obtained, indeed, through observations on certain features of the actions of autonomic nerves in particular, but in which the more recent and rapid advances of knowledge seem now to be spreading ever more widely, and well beyond any such strictly anatomical limitations or relationships. A glance at the contributions themselves has seemed to confirm these expectations of mine.

There are articles here, indeed, of great interest and importance, which deal with relatively recent developments of knowledge concerning the autonomic nervous system itself. Such are Professor Boyd's article on the " Intermediate sympathetic ganglia ", and that by Drs Murray & Thompson on " Collateral sprouting in response to injury in the autonomic nervous system and its consequences ". Both of these throw welcome new light on the nature of phenomena which it had earlier been difficult to explain, such as the anomalous persistence, or recovery, of peripheral sympathetic activities which an operative sympathectomy might have been expected to eliminate permanently; or the appearance, after injury or operation, of new and paradoxical associations of normally unrelated nervous activities. It should be noted, however, that this phenomenon, of side-branching from normal nerve fibres into degenerated fibre tracks, is by no means special to autonomic nerves. Professor Garry's article, again, on " The innervation of the abdominal viscera ", is concerned essentially with the autonomic nervous system; and he poses again, only to leave still without any clear promise of a solution, a problem which many have recognized, in the opposite responses given by different tracts of apparently similar involuntary muscle, not only to impulses in nerve fibres with the same anatomical connexion, but also to the artificial application of one and the same chemical transmitter of the effects of such impulses. Why should the same substance, whether naturally liberated or artificially applied, produce augmented activity in one case and inhibition in another? Here, surely, is one of the fundamental problems of physiology and pharmacology; and Boeke's postulation of an " interstitial cell net ", as cited by Professor Garry, appears to offer no real solution, but only to evade or defer the problem by transferring it hypothetically to another structure.

Professor Burn's article on " Acetylcholine and cardiac fibrillation " might seem to make some approach to this problem from a different angle, in demonstrating that one of the transmitters may produce on the same structure, cardiac muscle, what seems to be the direct opposite of its normal, inhibitory effect, when the organ has been reduced to an abnormal quiescence; but whether this phenomenon has any connexion with the contrasted actions on two otherwise similar structures, when both are under the same physiological conditions, is by no means clear.

Sir Charles Lovatt Evans might appear from the title of his contribution, " Sweating in relation to sympathetic innervation ", to be returning to a more literal interpretation of the general subject proposed for this symposium. In fact, however, it will be found that his article is largely concerned with the very interesting discovery, that the profusely active " apocrine " sweat glands of the horse are not directly innervated at all; so that their only relation to autonomic, or indeed to any nerves, is a very indirect one, in that they respond to adrenaline, not acting as an immediate transmitter of sympathetic nervous effects, but carried to them from the suprarenal medulla by the blood stream, and acting on them, therefore, as a true hormone, in the original sense in which that word was used by Bayliss and Starling. Is this, one wonders, the only case yet clearly revealed, in which an organ of an adult, higher vertebrate responds specifically, and physiologically, to a substance having elsewhere the function of a transmitter of autonomic nervous effects, but without possessing, or, apparently, having ever possessed, any corresponding innervation of its own? Maybe there are other such cases waiting to be discovered!

It may I think be regarded as inevitable, and, indeed, as entirely desirable, that contributors to a present-day symposium on autonomic nerves and their functions should find it impossible to accept a strict limitation to matters directly concerned with the prescribed subject. They cannot be expected to avoid dealing with the substances now long recognized as the chemical transmitters of the peripheral effects of impulses in autonomic nerves; and the occurrence and the functions of these substances have more recently acquired, in various directions, so

much wider a physiological interest and significance, that the title of the symposium inevitably comes to serve, for many of the contributory articles, as an appropriate peg for related ideas or problems, or as a point of departure for even wider explorations, rather than as a limiting framework. And so we have the article entitled " Acetylcholine metabolism at nerve-endings " by Dr Birks & Professor MacIntosh, in which we are not surprised to discover that the nerve-endings most convenient for studying this are not only, or even chiefly, those of autonomic nerves; or that by Dr Blaschko, in which he deals with the " Formation of catechol amines in the animal body " as a fundamental, biochemical problem; or that by Dr Marthe Vogt on " Sympathomimetic amines in the central nervous system ", in which she herself comments, with an engaging frankness, that " It may appear strange to include a review on the occurrence and distribution of the natural sympathomimetic amines in brain in a symposium on the autonomic nervous system "; and she proceeds to advance a more special plea of justification than the case appears to me to require. For Dr Bülbring's article on " The action of humoral transmitters on smooth muscle ", and for Dr Hutter's on the " Mode of action of autonomic transmitters on the heart ", it could, indeed, be argued, if it were necessary, that they deal with the nature of the responses of involuntary effector cells, to substances which normally transmit autonomic nervous effects; but it will be evident to their readers that these articles include considerations of the fundamental, physico-chemical mechanisms of such activities, of which the significance has no closely specific relation to the responses of involuntary effectors, or to autonomic stimuli.

Drs Hilton & Lewis, again, in their article on " Functional vasodilatation in the submandibular salivary gland ", are dealing with a phenomenon which is initiated, indeed, by impulses in autonomic nerves; but the aim of their article is to emphasize the extent to which, in their view, this vasodilatation is attributable, not directly to these autonomic nerve impulses and the immediate transmitters of their effects, but to the secondary formation, as the side-effect of a primary metabolic response, of a peptide vasodilator substance, which these authors do not regard as specifically associated with autonomic nervous activities. And while Drs Gray & Diamond, in their discussion of " Pharmacological properties of sensory receptors ", test the effects on these of the substances known, in other connexions, as transmitters of the effects of autonomic nerves, the sensory fibres to which these peripheral receptors belong are not, in any proper sense, " autonomic ", though some of them, of course, find their way to the central nervous system in autonomic nerves.

The proof-sheets of Dr Perry's stimulating review of the evidence concerning the " Transmission in autonomic ganglia " came late to hand, when I had already recorded my comments on the other contributions to this number of the Bulletin. His primary aim is to analyse this transmission process into its components, recognizing and reviewing, in the light of every detail of the available evidence, the nature of each successive link in the chain of events, from the initiation of an impulse in a preganglionic nerve fibre, to the issue from the ganglion of the corresponding small volley of postganglionic impulses. The ganglion furnishing these data certainly belongs to the autonomic system; in most cases, on account of its experimental convenience, it is the superior cervical ganglion of the cat. And Dr Perry is surely right in recognizing its special value to the experimenter as " a microcosmic nervous system ", in which the transmission process can be picked up for record at most of its stages, while the transmitter can be readily collected, identified and assayed in the venous effluent. All very true, of course. If we follow Dr Perry's picturesque conception of its microcosmic function, however, and inquire in relation to what macrocosm it provides him with suggestions of analogy, we find ourselves moving beyond and away from the autonomic system, which has provided, here again, an opening for entry into other fields of exploration. The transmission process in a ganglion certainly has analogies with that from a motor nerve-ending to the end-plate of a voluntary muscle fibre. It was, indeed, this analogy which first encouraged the inquiry, whether the voluntary nerve-to-muscle transmission might not also be cholinergic; and Dr Perry recognizes the relevance to his argument of some of the more recently discovered details of this latter process. And again, with every suitable warning against an application too close in its detail, the analogy with transmission at synapses in the central grey matter is too obvious to be ignored, and much too important to be minimized. The late Sir Charles Sherrington jumped to it at once, when I first told him of the evidence for the transmitter function of acetylcholine at voluntary nerve-muscle junctions.

And positive evidence for the value of that analogy is, of course, now being obtained, especially by the experiments of Professor Eccles and his co-workers in Canberra. On the other hand, the transmission process in autonomic ganglia, as analysed by Dr Perry, does not seem to teach us anything that is new about the more specifically autonomic transmission process, at the peripheral neuro-effector junctions with involuntary muscle and gland cells. For this we must look elsewhere in the articles composing this symposium; but I do not think that we shall find, in any of them, the real clue to what seems to me to be still the fundamental functional problem of the autonomic system—the action of the same transmitter, in producing augmentation in one case, and inhibition in another, of the spontaneous activities of tracts of involuntary muscle which, in all other respects, appear to be identical.

It has been my great privilege to watch for more than half a century, and at fairly close quarters, the development of the evidence for this transmission of excitation at neuro-effector and interneuronal junctions, by the liberation from nerve-endings of specific chemical transmitters. I was closely in touch with T. R. Elliott when, in 1904, with precociously brilliant insight, he made the first suggestion of such a process, for the special case of peripheral sympathetic nerve-endings and adrenaline. At that time, and for many years afterwards, the idea seemed to be too revolutionary for authoritative opinion to take it seriously; and it did not become really respectable till Otto Loewi, in 1921, produced the first convincing, experimental evidence in its favour. Since then the progress has been more rapid and continuous, though it was halted again for a period, while methods, courage and initiative were being acquired, for the experimental extension of the main transmitter conception, first to synapses in ganglia of the autonomic system, and then to analogous junctions beyond it. And now this number of *British Medical Bulletin*, reviewing what is known today about the autonomic nervous system and its functions, and following freely the leads offered by implication and analogy, gives the reader an exhilarating sense of the imminence of further developments, needing only special skill, allied with scientific vision and bold enterprise, to open new and ever widening vistas of knowledge, concerning junctional transmission in the nervous system as a whole.

Henry Dale

We wish to thank Professor J. H. Burn for acting as Chairman of the committee that planned this number of the Bulletin, and for all the trouble he took throughout.

ACETYLCHOLINE METABOLISM AT NERVE-ENDINGS

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The main peripheral cholinergic systems have now been recognized and their functional significance in most cases is fairly well understood; by contrast only fitful progress has been made in the identification of central cholinergic mechanisms. In recent years the focus of attention has shifted to the analysis of cholinergic transmission at the cellular level, i.e., to the mechanisms involved in the formation, storage, release and action of acetylcholine at synapses (including neuro-effector junctions). The present article deals with the first three of these, namely, synthesis, storage and release.

1. Synthesis of Acetylcholine

So far as we know, the acetylcholine of vertebrate tissues is synthesized entirely by a specific enzyme, choline acetylase (Nachmansohn & Machado, 1943), present in all cholinergic neurones (and in some other cells), and capable of transferring acetyl groups from coenzyme A (CoA) to choline (Feldberg & Mann, 1946; Lipmann & Kaplan, 1946; Nachmansohn & Berman, 1946). Theoretically acetylcholine can also be formed by a reversed action of the true, specific or acetyl cholinesterase, whose normal role is the hydrolytic destruction of acetylcholine, and whose cellular distribution is somewhat similar to that of choline acetylase, but it does not appear that conditions favouring this back-reaction are ever met with in vivo. Efficient synthesis of acetylcholine therefore requires the presence in adequate quantity of (i) choline acetylase, (ii) free choline, (iii) CoA, and (iv) active acetate for the formation of acetyl CoA. This last requirement can be fulfilled in a variety of ways, since carbohydrates, amino acids and fatty acids can all serve as sources of acetyl for interaction with CoA (cf. Novelli, 1953). The chemical energy required for the acetylation of CoA is usually made available in the form of adenosinetriphosphate (ATP); the common penultimate step may be the formation of adenyl acetate (Berg, 1955).

Choline acetylase. This enzyme occurs in very uneven concentration in different parts of the nervous system. As might be expected, its distribution closely parallels that of acetylcholine (Feldberg & Vogt, 1948). The best recent data (Hebb, 1955; Hebb & Silver, 1956) re-emphasize the contrasts noted by earlier workers, and strongly support the idea that the two substances do not occur in non-cholinergic neurones, but are present in substantial quantity throughout the axons of cholinergic nerves, as well as at their endings. It is generally supposed (cf. Feldberg & Vogt, 1948) that choline acetylase, like other neuronal enzymes, is manufactured in the cell body and is carried peripherally by movement of the axoplasm (Weiss, 1944). Support for this idea has recently been provided by Hebb & Waites (1956); they found that choline acetylase accumulates in the proximal stump of a severed cholinergic nerve during the days immediately following the section, when it is disappearing from the distal part of the nerve and from the nerve-endings. Further work by Hebb & Smallman (1956) has shown that most of the choline acetylase of brain is sedimented with the mitochondrial fraction in centrifuged sucrose homogenates. These results do not conclusively prove the mitochondrial location of the enzyme, since it is conceivable that in their experiments smaller cellular components became adherent to the mitochondria as a result of the fractionation procedures; the fractions however appeared on histological examination to contain no other structures, and moreover a number of other anabolic systems have been found to be predominantly localized in mitochondria. Some sort of subcellular particle, at any rate, must be particularly rich in choline acetylase; and Hebb and Smallman's finding, that their fractions exhibited full synthesizing activity only after treatment with ether, suggests that the enzyme may be located inside the particle rather than on its surface, and is for that reason relatively inaccessible to its substrates.

Whether acetylcholine can be stored in the cellular structures that manufacture it has not been demonstrated. Some insoluble cell constituent, at any rate, can bind acetylcholine quite firmly (Loewi & Hellauer, 1938; Trethewie, 1938). Recent developments in electron microscopy have focused attention on particles much smaller than the mitochondria, the so-called synaptic vesicles, which may be involved in the carriage of acetylcholine from the structures that synthesize it to the presynaptic membrane where it is set free. The evidence for this will be discussed below.

Choline. This substance is a normal constituent of the extracellular fluid, where its concentration is of the order of 10^{-5} M (Bligh, 1952). Presumably, however, it is the intracellular level of free choline that is significant in relation to acetylcholine synthesis, and about this not much is known. It appears that choline does not generally penetrate very readily into cells (Lorente de Nó, 1949; Bligh, 1953). In experiments on tissues containing intact nerve cells, for example, sliced or minced brain (Quastel, Tennenbaum & Wheatley, 1936; Mann, Tennenbaum & Quastel, 1938) or sympathetic ganglia perfused with saline solutions (Brown & Feldberg, 1936), the extracellular choline concentration appeared sometimes to be, and sometimes not to be, a limiting factor for acetylcholine production. This is perhaps not surprising, since the supply of active acetate in such preparations may well be sub-optimal for acetylcholine synthesis, and this rather than the intracellular choline level may be the rate-controlling factor. Even in severe choline deficiency, a reduction of tissue acetylcholine stores has not been clearly demonstrated (Best, 1956). Probably, then, a sufficient quantity of choline is normally available at the intracellular

sites of acetylation. Recent observations of our own strongly suggest, however, that the intracellular stocks of choline are scanty, and must, when acetylcholine turnover is brisk, be continually replenished from the extracellular fluid. Evidence for this comes from experiments with a " hemicholinium " base HC3 (Schueler, 1955), which strongly depresses acetylcholine synthesis by intact neurones (MacIntosh, Birks & Sastry, 1956), almost certainly by competing with choline for a specific carrier located in the cell membrane (Birks, unpublished). The existence of such a choline-transport mechanism makes it easier to understand the observation (Birks, unpublished) that during prolonged activity a sympathetic ganglion can convert into acetylcholine—and release —as much as 30% of the choline carried to it by the blood. The presynaptic endings could hardly maintain such a rate of choline turnover unless they were equipped with some kind of special portal for the ingress of choline ions.

Coenzyme A and " active acetate ". CoA appears to be a ubiquitous constituent of cells (Novelli, 1953), as would be expected from its key position at the intersection of many metabolic pathways. It is probably never a limiting factor for acetylcholine synthesis in vivo, except perhaps when its tissue levels have been greatly reduced as a result of pantothenic acid deficiency (Novelli, 1953; Bose, Gupta & De, 1954).

The " active acetate " required for the formation of acetyl CoA can be supplied, as already noted, from a wide variety of intracellular sources. A full discussion of the biochemical mechanisms involved would be outside the scope of this article. In cell-free systems either acetate or citrate can efficiently supply the two-carbon fragment (Balfour & Hebb, 1952). Intact neurones, on the other hand, require an external source of glucose for the support of acetylcholine synthesis (Quastel *et al.* 1936; Kahlson & MacIntosh, 1939; Crossland, Elliott & Pappius, 1955); lactate or pyruvate can replace glucose, but acetate and citrate can not. For optimal synthesis, and for prolonged maintenance of the full acetylcholine output, the extracellular fluid must contain some other organic substance besides glucose and choline: this is an unidentified heat-labile factor present in plasma dialysates (Birks & MacIntosh, unpublished). Glucose and the plasma factor are probably significant in relation to the activation rather than to the formation of the two-carbon fragment; it is doubtful that acetylcholine synthesis in vivo is ever limited by the supply of acetate.

2. Storage of Acetylcholine

Brown & Feldberg (1936) found in their pioneer studies of acetylcholine metabolism that the amount of acetylcholine they could extract from a sympathetic ganglion appeared to have little relation to the ganglion's immediate past history. For when a ganglion, perfused with Locke's solution containing glucose and eserine, was stimulated through its preganglionic trunk for 30 min. or more, its output of acetylcholine progressively declined, as already noted, to a low steady level and could be only partly restored by rest; yet such a ganglion, when extracted, yielded about as much acetylcholine as a ganglion that had never been stimulated. Synthesis, it seemed, had kept pace with release in the stimulated ganglion, but the newly-formed acetylcholine was less easily discharged by nerve impulses. Perry (1953) explained these and other findings by postulating that the acetylcholine of the presynaptic endings exists in two forms, one available for immediate release and one not so available; the unavailable form, he believed, can be made available at a slow steady rate which is independent of the parameters of stimulation. He also suggested, on the basis of indirect evidence, that the presynaptic endings could take up most of the choline derived from the breakdown of the released transmitter, and could use it for the synthesis of new available acetylcholine; extracellular acetylcholine protected from hydrolysis by eserine could not be reclaimed in this way.[1]

Perry's hypothesis of two forms of acetylcholine remains an attractive one, but needs some modification in the light of newer observations (Birks & MacIntosh, unpublished experiments). In ganglia that are perfused with eserinized plasma rather than with eserinized Locke's solution containing glucose, and can thus synthesize acetylcholine much more efficiently, the extractable acetylcholine rises progressively during an hour to about double the normal level, whether or not the presynaptic fibres are being stimulated. If the perfusion is then continued with eserine-free plasma, this surplus acetylcholine quickly disappears. The surplus acetylcholine must therefore have accumulated in spaces which in the absence of eserine contain active cholinesterase, and which are not external to the presynaptic terminals; for, in the absence of stimulation, no detectable acetylcholine leaks into the eserinized effluent. The " surplus " is apparently not " available " in Perry's sense, since, as already mentioned, the output of acetylcholine from a ganglion perfused with eserinized plasma does not rise along with the acetylcholine content, but is maintained indefinitely at a steady level. The acetylcholine contained in ganglia that have been perfused with eserinized Locke's solution and stimulated for a long time is probably for the most part surplus acetylcholine which can be neither released, nor de-esterified so that it can be used to make new available acetylcholine.

The simplest way to account for all these findings is to suppose that the surplus acetylcholine is free in the presynaptic axoplasm and that the available acetylcholine is held within subcellular particles of some sort. The idea that presynaptic acetylcholine is released from a particulate store has already been reached, on quite different grounds, by other investigators (for references see Castillo & Katz, 1956 and Eccles, 1957), most of whom have identified the acetylcholine-containing particles with the minute vesicles, of diameter about 40 mμ., which appear from electron micrographs to abound in the presynaptic axoplasm of all junctions so far examined (Palade & Palay, 1954; de Robertis & Bennett, 1955; Robertson, 1956). Katz and his co-workers (Fatt & Katz, 1952; Castillo & Katz, 1956), whose beautiful studies have done most to elucidate this problem, have shown that the end-plates of resting amphibian and mammalian muscle normally exhibit small fluctuations of membrane potential which can be due only to the spontaneous release of " packets " or " quanta " of acetylcholine, random in time but roughly equal in size, and each containing hundreds or thousands of acetylcholine molecules (Fatt & Katz, 1952). The normal end-plate potential, which triggers the propagated response of a muscle fibre, is undoubtedly set up by the nearly simultaneous release of a great number of such quanta (Castillo & Katz, 1954b; Martin, 1955). These quantal phenomena have so far been seen only at the neuromuscular junction, but it seems highly probable that

[1] See also Perry, p. 220 of this number of the Bulletin.—Ed.

they occur at all cholinergic endings, and indeed at synapses generally.

The idea just discussed, that the extractable acetylcholine of nervous tissue is located mainly in the vesicular elements that characterize the presynaptic axoplasm, introduces certain difficulties. Nerve axons, for example, seem to be free of these elements (Robertson, 1956). Yet cholinergic fibres may contain about as much acetylcholine, weight for weight, as ganglia (MacIntosh, 1941), and under certain circumstances may release a little of it into an external medium in response to stimulation (Lissák, 1939). These findings might appear to rule out the synaptic vesicles as carriers of acetylcholine. Since, however, the synaptic endings account for most of a ganglion's acetylcholine but probably for no more than 1 % of its volume, they must contain acetylcholine (and acetylcholine-carrying particles) in far higher concentration than the axons from which they are derived. The acetylcholine of the axons may perhaps be held in vesicles so sparsely scattered that they have been overlooked by electron micrographers. Another possibility is that the acetylcholine of the axons is all retained within the mitochondria or other structures that manufacture it, and that it is only in the synaptic terminals that these load their product into the smaller vesicles which deliver it to the presynaptic membrane.

On balance, then, the evidence, while far from conclusive, suggests that two kinds of particle are involved in the supply of acetylcholine to the releasing mechanism: one, perhaps identical with the mitochondrion, whose function is mainly related to synthesis of acetylcholine, and another, which may be the synaptic vesicle, concerned with its storage and release. How these two are linked can only be guessed: possibly the vesicles originate from the endoplasmic reticulum (Palade, 1956; Hodge, McLean & Mercer, 1956) which is in turn formed as a result of mitochondrial acuivity (Bernhard & Rouiller, 1956). Once formed, the vesicles can presumably reach the presynaptic membrane as a result of their random thermal movement; no special transport mechanism would seem to be required.

Whatever may be the form of the particle in which acetylcholine is carried, the total amount of acetylcholine that can be stored within a neurone is no doubt limited by the capacity of the carrier particles rather than by the neurone's ability to synthesize acetylcholine. Resting nerve-endings, which contain their full complement of acetylcholine, continue to manufacture and release it, but far more slowly than they do when they are active. Presumably, when activity ceases, the formation of acetylcholine and the formation of carrier material are both sharply reduced as soon as the depleted transmitter stores have been replenished. It is hard to guess how this braking action is applied to the transmitter " production line ": it can hardly be due either to exhaustion of raw material, or to a mass-action effect resulting from accumulation of the finished product (unless all the available space in the axoplasm is filled with it). A similar difficulty exists in connexion with other specific cell products, such as the secretory granules of exocrine gland cells.

3. Release of Acetylcholine

For any acetylcholine-containing particle within a presynaptic ending, the probability that it will discharge its transmitter store within the next few milliseconds is extremely small. The probability is greatly increased, though still small (in a sympathetic ganglion it is about 10^{-4}), when the ending during that time is invaded by a nerve impulse. So far the facts are well established. It is still far from clear, however, just what change in the particle's environment, consequent on the arrival of the impulse, makes it more likely that the particle will be mobilized and will unload its acetylcholine into the extracellular space. Presumably the particle must fuse at least temporarily with the membrane: it will not suffice that the particle should merely rupture at the membrane's inner surface; for in that case, the acetylcholine that accumulates in eserinized nerve-endings should be available for release by the nerve impulse, but it is not. The local potential gradients associated with depolarization can hardly be steep enough to move the particles, supposing that these are charged, more than a small fraction of their diameter in the time available. It is easier to imagine that the fusion of particles and membrane may be facilitated by a local diminution of axoplasmic viscosity that would permit increased Brownian movement of particles, or by some modification of the surface properties of either particle or membrane that would lead to diminution of short-range repulsive forces between them. Such changes as these might result from a modification of the ionic composition of the axoplasm close to the membrane, such as is known to occur in axons when they become depolarized, or to an alteration in the membrane itself.

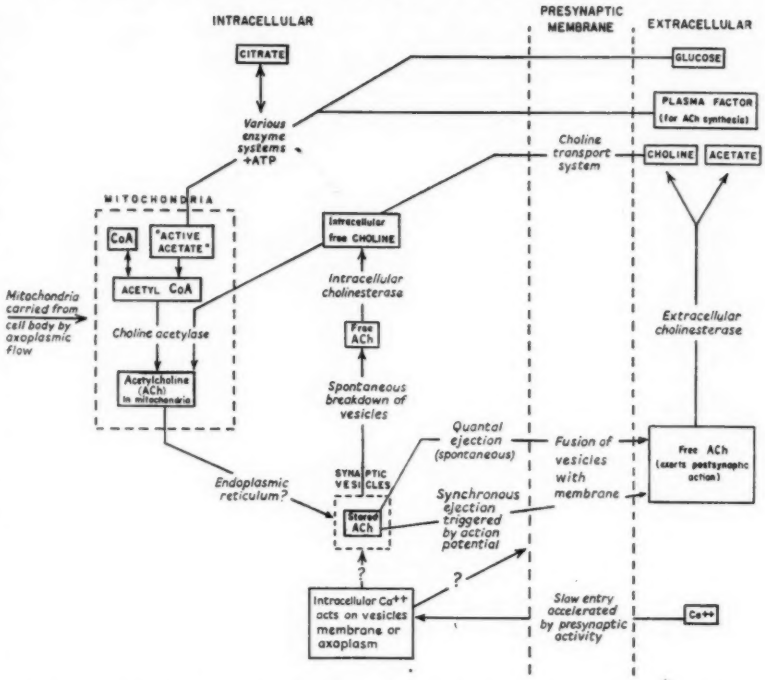
If it is an ionic change in the axoplasm that triggers the release of acetylcholine, this change can hardly be the entry of sodium ions, since the amount of acetylcholine liberated from a stimulated ganglion is independent of the external sodium level over a wide range (Hutter & Kostial, 1955); moreover both the spontaneous quantal release of acetylcholine, and the more copious outpouring that occurs when the endings are depolarized by a rise of external potassium, can occur in the complete absence of sodium (Castillo & Katz, 1955; Hutter & Kostial, 1955). It is even less likely that movements of potassium ions are responsible: the release of acetylcholine by application of potassium is clearly secondary to the depolarization this produces. A more attractive hypothesis would be that it is the inward movement of calcium ions (Flückiger & Keynes, 1955) that mobilizes the acetylcholine units. The presence of calcium in the extracellular fluid is indispensable for the release of acetylcholine both by presynaptic impulses and by potassium (Harvey & MacIntosh, 1940); when the calcium concentration is changed, there is a corresponding and striking change in both the total amount of acetylcholine discharged by each impulse (Hutter & Kostial, 1954), and in the number of quanta contributing to the discharge at each junction (Fatt & Katz, 1952; Castillo & Katz, 1954b). In an equally remarkable way, magnesium depresses acetylcholine release and antagonizes the junctional effects of calcium (Castillo & Engbaek, 1954; Castillo & Katz, 1954a; Hutter & Kostial, 1954). One difficulty about supposing that the entry of calcium is directly responsible for mobilizing the acetylcholine units is that the spontaneous release of acetylcholine at frog motor-nerve endings is little affected by changes in external calcium or magnesium (Fatt & Katz, 1952). Quite possibly, however, it is the intracellular level of calcium that matters; this is apparently always low (Keynes & Lewis, 1956), and it may be nearly independent of the external level except where a temporary depolarization facilitates entry of these ions. Calcium ions that enter an axon are extruded only very slowly (Flückiger & Keynes, 1955), and if the same is true for nerve-endings, a rise of intracellular calcium might account for the increased acetylcholine release, both resting

and triggered by excitation, that follows repetitive activation of the endings (Liley & North, 1953; Castillo & Katz, 1954c; Liley, 1956a, 1956b). At present the biggest obstacle to any theory relating the lability of the acetylcholine stores to the internal calcium level would be the finding (Flückiger & Keynes, 1955) that in *Loligo* axons the activated calcium influx appears to be only about six times the resting influx: to meet this objection some adjuvant hypothesis would have to be constructed. Further information about the factors controlling calcium (and magnesium) fluxes at nerve-endings may clarify the situation.

Various other procedures are known to affect acetylcholine liberation, both spontaneous and induced by excitation, but their effects have not as yet helped very much in elucidating the mechanisms of release. Among these may be mentioned raising the temperature (Fatt & Katz, 1952; Brown, 1954; Kostial & Vouk, 1956), which can facilitate both kinds of release; application of botulinum toxin, which stops both kinds (Brooks, 1956); moderate anodal polarization of the presynaptic endings, which increases the nerve-induced release without much effect on the spontaneous release (Castillo & Katz, 1954d); and raising the osmotic pressure, which greatly increases the frequency of the spontaneous discharge (Fatt & Katz, 1952; Boyd & Martin, 1956; Furshpan, 1956).

In addition repetitive presynaptic excitation may, as we have seen, lead to reduced acetylcholine output, with accompanying "fatigue" of transmission when the extracellular medium is deficient in the materials required for optimal synthesis. When these materials are supplied, as in a ganglion perfused with plasma, we find (Birks, unpublished) that the acetylcholine output per preganglionic volley is independent of the stimulation frequency over the range 2–20/sec.; at higher frequencies than these it is the output per minute that is constant, while the output per volley varies inversely with the frequency and is poorly synchronized with the stimuli (cf. Castillo & Katz, 1954c); only at very low frequencies, 1/sec. or below, is the volley output higher than over the middle range of frequencies. Such results as these suggest that at frequencies below 20/sec. the time between impulses is under normal circumstances sufficient for restitution of the available acetylcholine; at higher frequencies the release is limited by the rate at which the stores can be made available, this limiting rate being, perhaps, the rate at which synaptic vesicles move up to the inner surface of the membrane. It seems improbable that junctional transmission is ever impaired, under physiological conditions, because there is a shortage of available acetylcholine at the nerve-endings. Prolonged high-frequency excitation tends to cause excessive rather than deficient post-synaptic action; if block occurs, it is because the transmitter is released asynchronously and the post-synaptic membrane has no opportunity to recover from its effects.

FIG. 1. SCHEMATIC REPRESENTATION OF THE PROBABLE COURSE OF ACETYLCHOLINE METABOLISM AT NERVE-ENDINGS



Processes involved in acetylcholine synthesis are shown in upper part of diagram; processes involved in acetylcholine storage and release are shown in lower part. Broken lines represent phase boundaries.

4. Conclusions

The findings discussed above permit the construction of a tentative scheme (fig. 1) that would be compatible with what we know about acetylcholine metabolism in cholinergic neurones. In brief, mitochondria manufactured in the perikaryon are carried by axoplasmic flow to the nerve-endings. They contain choline acetylase, and contain or can synthesize CoA, which becomes acetylated most efficiently when carbohydrate is being aerobically utilized. Choline enters the nerve-endings by a special transport mechanism, and acetyl groups are transferred to it from CoA by the mitochondrial choline acetylase; the acetylcholine thus formed is loaded by mechanisms unknown into small carrier-particles, the synaptic vesicles, which reach the presynaptic membrane by Brownian movement. A unit (some hundreds or thousands of molecules) of acetylcholine is released, and a unit of post-synaptic action occurs, whenever a vesicle makes an effective collision with the presynaptic membrane. Such effective collisions occur singly at random intervals when the membrane is at rest, more frequently when it has recently been excited, and synchronously in large numbers when it is depolarized by a nerve impulse. The problem of what determines an effective collision is unsolved: the calcium concentration of the presynaptic axoplasm may be an important factor.

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FORMATION OF CATECHOL AMINES IN THE ANIMAL BODY

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1 Intermediate stages in the formation of adrenaline and noradrenaline
  a The pathway of formation
  b L-Dopa decarboxylase
  c Formation of noradrenaline from dopamine
  d Methylation of noradrenaline
  e Catechol amine formation in the intact animal
2 Intracellular localization of the formation of catechol amines
3 Intermediary metabolism and storage of amines
4 Conclusions
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The close chemical and pharmacological relationship between chromaffin tissue and the adrenergic neurone had been discussed by Elliott (1904) when the idea of chemical transmission of nervous impulses was still in its infancy. This idea was placed on a secure basis through the discovery of the "Acceleransstoff" (see Loewi, 1921). Today adrenaline and noradrenaline are considered as the two hormones released by chromaffin tissue, and noradrenaline as the principal mediator of adrenergic neurones (von Euler, 1956). For the study of the formation of these two catechol amines, the medulla of the suprarenal gland has been the most convenient material, as it is easily accessible and rich in amines; a few biochemical studies on adrenergic neurones seem to show that amine formation in chromaffin tissue and sympathetic neurones follow similar lines.

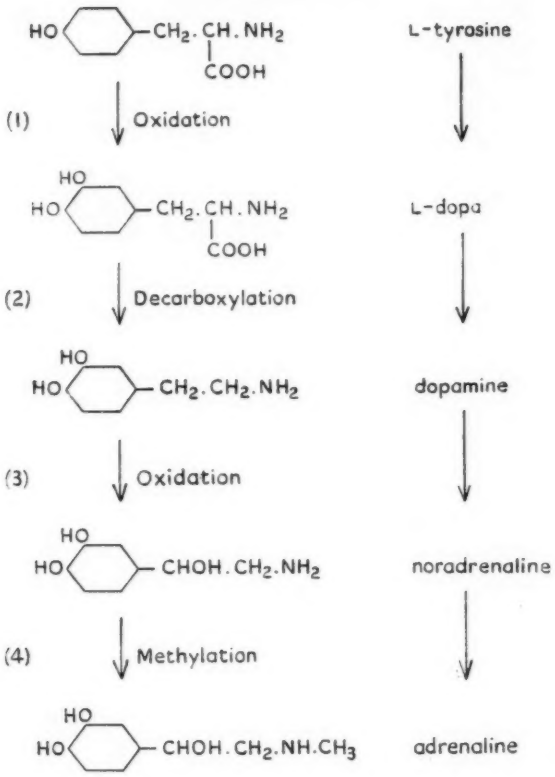
The structural similarity between adrenaline and the amino acids phenylalanine and tyrosine was noted at an early stage, and the amino acid now known as dopa (3:4-dihydroxyphenylalanine) was first prepared at the Lister Institute, London, by Casimir Funk, because he believed it to be an intermediate in the formation of adrenaline in the organism; he was, however, not able to obtain experimental evidence in support of his belief (Funk, 1911).

1. Intermediate Stages in the Formation of Adrenaline and Noradrenaline

a. The Pathway of Formation

Many pathways of adrenaline formation from tyrosine are theoretically possible, but in the following section one will be mainly discussed, because recent work has brought forward support for it. The scheme given in fig. 1 arises from a suggestion (Blaschko, 1939) that the enzyme dopa decarboxylase was the catalyst of one of the reactions that lead from tyrosine to adrenaline.

FIG. 1. INTERMEDIATE STAGES IN THE FORMATION OF ADRENALINE



b. L-Dopa Decarboxylase

This enzyme was discovered by Holtz, Heise & Lüdtke (1938) in the mammalian kidney, but it was only in 1950 that Langemann showed in this Department that the bovine adrenal medulla was a rich source of dopa decarboxylase (Langemann, 1951). This finding, since confirmed for other vertebrate species, including man, gave the first definite proof of the ability of chromaffin tissue to form a sympathomimetic amine by enzymic action; it suggested that step (2) of the scheme actually occurred in this tissue. Recent work of Holtz & Westermann (1956) has shown that nervous tissue, in particular the adrenergic neurones, also contains dopa decarboxylase. These authors survey the enzymic activity of different parts of the nervous system, and their finding that the hypothalamus has relatively high activity is of interest in connexion with the occurrence of brain "sympathin" in this locality (discussed in the contribution by Dr Marthe Vogt, p. 166 of this number of the Bulletin); it seems, therefore, likely that brain sympathin is actually formed in the hypothalamus.

The demonstration of dopa decarboxylase in the adrenal medulla was followed by the finding that in some species, especially in cattle and in sheep, this tissue also contains demonstrable amounts of dopamine (Goodall, 1951). Here again, the adrenergic neurone is similar to the chromaffin

tissue: Schümann (1956) has recently found dopamine in the splenic nerve of the ox. Whereas in the adrenal medulla dopamine contributes only a small percentage of the total catechol amines, the amounts of dopamine in adrenergic neurones represent approximately one-half of the total catechol amines; the other half is noradrenaline. Dopamine is also present in human urine (von Euler, Hamberg & Hellner, 1951).

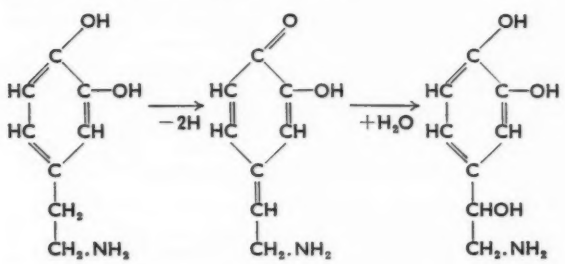
c. Formation of Noradrenaline from Dopamine

The next step was to study the formation of noradrenaline. This was first undertaken in collaboration with Dr A. D. Welch, now Professor of Pharmacology of Yale University, who came to Oxford as a Senior Fulbright Research Fellow in 1952. Homogenates of ox suprarenal medulla were prepared, but in the course of preliminary studies it was found difficult to free such homogenates from catechol amines. This finding led to the observation that the bulk of the adrenaline and noradrenaline was present in granules which do not readily give up the amines (Blaschko & Welch, 1953). It was therefore decided to use for the study of noradrenaline formation isotopically labelled precursors. Work carried out at Yale University in 1953–54 showed that when DL-dopa-2 ^{14}C (DL-dihydroxyphenyl-[2-^{14}C]-alanine) was incubated with bovine adrenal homogenates, fortified by the addition of co-factors, almost 50% of the radioactivity was present as dopamine, i.e., most of the radioactivity present in the L-dopa; a small amount of radioactive noradrenaline had also been formed (Demis, Blaschko & Welch, 1956).

This work has since been followed up by Hagen (1956); he isolated the radioactive dopamine formed in the incubation with bovine homogenates and re-incubated it with a homogenate from the suprarenal gland of the domestic fowl. In this way evidence for the occurrence of step (3) of the scheme in fig. 1, the direct formation of noradrenaline from dopamine, was obtained (see also Hagen & Welch, 1956).

More recently, Hagen's results on noradrenaline formation from dopamine have been supplemented by observations on acetone-dried preparations of bovine adrenal medulla (Neri, Hayano, Stone, Dorfman & Elmadjian, 1956); these preparations, when incubated with dopamine in the presence of co-factors (adenosinetriphosphate (ATP) and either diphospho- or triphosphopyridine nucleotide), gave rise to a noradrenaline-like material.

The mechanism of the introduction of the aliphatic hydroxyl group is not yet known; the reaction can be formulated as a dehydrogenation reaction, with subsequent addition of H_2O. A possible formulation is given below:



Alternative formulations have been discussed in the analogous oxidation of ethylbenzene to methylphenylcarbinol: $Ph.CH_2.CH_3 \longrightarrow Ph.CHOH.CH_3$, with the intermediate formation of either a free radical, $Ph.\dot{C}H.CH_3$, or a carbonium ion, $Ph.\overset{+}{C}H.CH_3$ (Smith, Smithies & Williams, 1954).

Recent observations on slices of cat adrenal medulla have brought support for the role of dopamine as a precursor of noradrenaline (and adrenaline): Kirshner & Goodall (1956) have studied the formation of catechol amines from ^{14}C-labelled tyrosine, using an Amberlite column for separation of the amines and the characteristic extinction in the ultraviolet (at 280 mμ.) for their determination. It was found that upon incubation with tyrosine the radioactivity first rose in the dopamine, subsequently in the noradrenaline, and only after some hours of incubation radioactivity slowly appeared in the adrenaline.

d. Methylation of Noradrenaline

Evidence for the formation of adrenaline from noradrenaline was first obtained in 1949 by Dr Edith Bülbring in experiments in which the ground suprarenal gland of a dog was incubated in vitro; an increase in adrenaline at the expense of the noradrenaline initially present was observed when ATP was added during the incubation. The increase in adrenaline was most pronounced when the glands had been removed from the animals after the splanchnic nerve supplying the gland had been stimulated.

e. Catechol Amine Formation in the Intact Animal

Radioactive precursors have been extensively used in these studies. Gurin & Delluva (1947) administered labelled phenylalanine to rats and recovered radioactive adrenaline from the suprarenal glands. A few years later, Udenfriend, Cooper, Clark & Baer (1953) found that ^{14}C-labelled phenylalanine or tyrosine, when administered intraperitoneally to rats or rabbits, gave rise to radioactive adrenaline. More recently, Udenfriend & Wyngaarden (1956) have administered a series of radioactive substances and tested the incorporation of radioactive carbon into the medullary adrenaline and noradrenaline. It was established that dopamine was most readily incorporated, then followed dopa and then tyrosine. No incorporation occurred when ^{14}C-labelled tyramine or β-phenylethylamine was administered.

The introduction of the *N*-methyl group of adrenaline has also been studied with the help of radioactive precursors. Keller, Boissonnass & du Vigneaud (1950), e.g., have shown that the methyl group of ^{14}C-labelled methionine can be transferred to adrenaline isolated from the adrenal gland of the rat. However, the recent work of Verly (1956) with methionine doubly labelled (with both ^{14}C and tritium) in the methyl group makes it doubtful if the methyl group is transferred intact, as the ^{14}C:tritium ratio in the methyl group of the adrenaline isolated from the rat's suprarenal gland differed from that in the methionine administered. On the other hand, in the choline and creatine isolated from the same animals, the ^{14}C:tritium ratio was the same as in the administered methionine; this is what would be expected if the $—CH_3$ group were transferred in toto.

The general enzymic mechanism of methyl group transfer has recently been studied by Cantoni (1956): the immediate methyl donor is "active" methionine, i.e. *S*-adenosylmethionine. It is at present not known if this or a similar compound plays a part in the methylation of noradrenaline. However, it is of interest to note that the methylation reaction

studied by Cantoni requires an enzymic removal of the phosphate groups from ATP. A very active ATPase is present in the adrenal medulla (Zeller, 1948). The localization of phosphatase in chromaffin tissue has been studied by Eränkö (1952) and by Hillarp & Falck (1956).

The site and the mechanism of the introduction of the phenolic hydroxyl group, step (1) of the scheme, is also not yet clear. The work of Udenfriend & Wyngaarden (1956) already mentioned shows that dopa can be converted to adrenaline and noradrenaline when it reaches the chromaffin cell from without; it seems likely, however, that the chromaffin cell is also able to make dopa. Dopa is the common precursor of melanin and the catechol amines, and it has been pointed out by Willmer (1951) that in vertebrates the formation of L-dopa from L-tyrosine seems to be a common characteristic of cells derived from the neural crest: melanoblasts, chromaffin cells and adrenergic neurones.

Summing up, it can be said that studies both of the enzymic reactions in vitro as well as of catechol amine formation in the living animal make it likely that the sequence of reactions given above represents the main pathway of formation of adrenaline and noradrenaline. It must be emphasized, however, that much work remains to be done. Of the enzymes responsible for the different steps, only one is known; this is dopa decarboxylase. Also, the rate of synthesis of the catechol amines is still under discussion. In the work of Udenfriend *et al.* (1953), the rate of synthesis was found to be slow. Hagen & Welch (1956) have pointed out that the method used measures the rate of synthesis of the amine stored but not necessarily that of the amine released; the recent work by Holland & Schümann (1956) on the effect of splanchnic stimulation upon output and storage of catechol amines suggests that the rate of their formation in the stimulated gland is fast.

2. Intracellular Localization of the Formation of Catechol Amines

In recent years, methods have been devised which allow the isolation of cytoplasmic particles from chromaffin tissue that store adrenaline and noradrenaline in very high concentration; similar methods were developed independently and at the same time in Oxford and Lund (Blaschko & Welch, 1953; Blaschko, Hagen & Welch, 1955; Hillarp & Nilson, 1954). The chromaffin granules represent the main storage sites of the two amines in the adrenal medulla. Recent observations on the localization of noradrenaline in the splenic nerve of the ox make it likely that in adrenergic neurones the mediator is also stored in granules (von Euler & Hillarp, 1956).

Are the chromaffin granules the sites of amine formation as well as of storage? In view of what has been said in the preceding section, this question will have to be answered separately for each of the reactions given in the scheme of biosynthesis.

There is at present no indication that the formation of dopamine occurs in granules. In homogenates of adrenal medulla, dopa decarboxylase has been found in the supernatant fluid. Provisionally, therefore, it is assumed that the formation of dopamine in chromaffin tissue (and in other tissues with dopa decarboxylase activity) takes place in the cytoplasmic sap and not in granules.

The intracellular site of formation of noradrenaline has not been studied. The occurrence of this reaction (step 3) in adrenergic neurones remains to be demonstrated.

During the last few years histochemical evidence has been given for the presence in chromaffin tissue of two types of cells (Bänder, 1950; Hillarp & Hökfelt, 1953; Eränkö, 1955); one of these stores mainly adrenaline, the other mainly noradrenaline. If two cell types exist, it must be assumed that they differ in that one of them is fitted out with a mechanism for methylating noradrenaline, whereas the other is not.

When chromaffin granules are prepared and suspended in approximately isotonic sucrose solution (0.3 M), adrenaline and noradrenaline are sedimented in a similar way when the suspension is centrifuged. When the granules are centrifuged in a density gradient over hypertonic sucrose solution, most of the granules are sedimented in a 1.5 M-sucrose solution; but only a small fraction of the granules is sedimented when the concentration of the sucrose is raised to 2.0 M (Blaschko, Born, D'Iorio & Eade, 1956). Eade (1956) has shown that the sediment obtained, when a suspension of granules from bovine adrenal medulla is centrifuged in 0.3 M-sucrose at high speed over 2.0 M-sucrose, has a higher noradrenaline content than the granule fraction from which it was derived; a fraction with a higher percentage of adrenaline content is retained in suspension in the higher parts of the centrifuge tube. These experiments have recently been extended by Schümann (1957), who used a density gradient method of centrifugation in the study of the distribution of adrenaline and noradrenaline in the adrenal gland of the domestic fowl. In his experiments, the uppermost layers of the density gradient tubes consisted of granules very poor in catechol amines; the intermediate layers contained a suspension of granules which stored only adrenaline. The sedimented granules contained a mixture of adrenaline and noradrenaline. Careful re-centrifugation of the re-suspended sediments yielded a small sediment that contained almost only noradrenaline. In these experiments, there always remained a fraction that contained both adrenaline and noradrenaline. The method employed does not tell us whether this fraction represents an incompletely resolved mixture of two types of granule, each storing only one amine, or whether there exists a third type which carries both adrenaline and noradrenaline.

The density gradient method of centrifugation has also made it possible to give an answer to the question whether or not the chromaffin granules are mitochondria. Recent work on granule suspensions from the ox medulla has shown that the granules held back in the upper layers of the density gradient tube, which are almost free of amines, contain the typical mitochondrial enzymes, succinic dehydrogenase and fumarase; the granular fractions rich in catechol amines are poor in these enzymes (observations by Blaschko, Hagen & Hagen, 1957). This finding is of interest, as evidence has been given by the electron microscope of the presence in the chromaffin cell of two types of cytoplasmic structure: typical mitochondria and strongly osmiophilic granules; the latter probably represent the amine-carrying (chromaffin) granules (Lever, 1955; Sjöstrand & Wetzstein, 1956).

3. Intermediary Metabolism and Storage of Amines

If a substance E is derived from an ultimate precursor A by a number of intermediate stages: $A \rightarrow B \rightarrow C \rightarrow D \rightarrow E$, one might expect to find conditions in which the formation of one of the earlier intermediates is normal, whereas a later stage is absent or reduced. In such conditions one of the earlier intermediates may accumulate, whereas the later-stage

substance is not found at all or in smaller amounts. Such conditions pertain during growth. Holton (1951) has found in the suprarenal gland of new-born calves a preponderance of noradrenaline. Shepherd & West (1952), who have made a detailed study of this change-over from noradrenaline to adrenaline during growth, have found only noradrenaline in the organ of Zuckerkandl, which in man disappears early in infancy. This organ never seems to acquire the ability to methylate noradrenaline.

A similar condition of metabolic block occurs in tumours of the chromaffin tissue, which are often extremely rich in noradrenaline (Holton, 1949), and recently a chromaffin tumour in man has been described in which dopamine, not normally stored in human chromaffin tissue, was the principal catechol amine present (McMillan, 1956); in another case, reported by Weil-Malherbe (1956), more than one-third of the total catechol amines was dopamine.

These observations support the scheme of reactions outlined on p. 162. However, it must be borne in mind that alternate pathways of formation of adrenaline and noradrenaline may exist; some of these, for example, from dihydroxyphenylserine or from octopamine, have been discussed elsewhere (Blaschko, Burn & Langemann, 1950). The possibility that a different pathway of formation of the catechol amines may exist in cephalopods is supported by experimental evidence, as octopods contain in their posterior salivary glands tyramine and the closely related octopamine, substances not found in mammalian tissues:

tyramine octopamine

Mention has already been made of the fact that in most mammals the enzyme dopa decarboxylase occurs in large amounts also in the liver and in the kidneys. If the chromaffin tissue and the adrenergic neurones are able to make their own dopamine, then the presence of the enzyme in other tissues may point to a physiological significance of catechol amines in these tissues of which at present nothing is known.

4. Conclusions

In a recent review on neurohormones, Welsh (1955) writes: "The exact pathways in the synthesis of nor-adrenaline and adrenaline are not known". Whereas only two years ago this statement gave a true account of the position, today the pathways of formation of the catechol amines are no longer unknown. Some new findings have been discussed in the preceding pages. There still remain many gaps in our knowledge of amine formation; some of these have also been pointed out in this review.

In his article, Welsh refers to the recognition that secreting and nervous tissue are functionally related. This relationship has been fully confirmed by the new findings; these have shown that the chromaffin cell and the adrenergic neurone both are the seat of specific chemical abilities which serve the main function of these tissues: elaboration, storage and release of catechol amines. The close biochemical relationship is in harmony with the histogenetic and pharmacological relations between adrenal medulla and sympathetic nervous system which have been known for a long time. We see in these new findings fresh support for the idea that release of adrenal medullary hormones and liberation of the adrenergic mediator are fundamentally similar events.

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SYMPATHOMIMETIC AMINES IN THE CENTRAL NERVOUS SYSTEM

Normal Distribution and Changes Produced by Drugs

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1 The term " sympathin "
2 Estimation of sympathin in brain
3 Distribution of sympathin in brain
4 Action of drugs on sympathin in brain
5 Comparison of the effects of drugs on sympathin content of the hypothalamus and on activity of the sympathetic centres
6 Conclusions
References

The question of the distribution of noradrenaline and adrenaline in the brain is part of the general problem of mapping the concentrations of substances which do not occur throughout the whole white or grey matter, but for which regions of abundance as well as regions of paucity (or complete lack) exist. The interest in such unevenly distributed compounds lies in the fact that they may be expected to play a role in some specialized function of the regions in which they abound. Thus the high content of choline acetylase in the anterior roots, and its absence in the pyramidal tract, suggest that only the first of these neurones transmits its impulses by the release of acetylcholine (Feldberg & Vogt, 1948), and the occurrence of vasopressor or oxytocic activity in the supra-optic and paraventricular nuclei singles these areas out as the sites of manufacture of what were once considered to be hormones elaborated by the posterior lobe of the pituitary.

It may appear strange to include a review on the occurrence and distribution of the natural sympathomimetic amines in brain in a symposium on the autonomic nervous system. There are, however, two reasons which justify this inclusion: one is that knowledge about metabolism and function of these amines in one part of the body may help in understanding their role in another part, and the second is that those parts of the brain which perform certain autonomic functions are also those which are particularly rich in these amines (Vogt, 1954).

1. The Term " Sympathin "

Sympathetic ganglia and postganglionic sympathetic nerves contain a mixture of noradrenaline and its methylated derivative adrenaline, the latter forming about 3% of the total. Peart (1949) has found that stimulation of the splenic nerves liberates essentially noradrenaline; in some experiments 1–2% of adrenaline was found to be also released. It is thus possible that the two amines are always released simultaneously on stimulation, though the amounts of adrenaline are so small that they may remain undetected. The mixture of large quantities of noradrenaline with a little adrenaline found in the peripheral sympathetic system may be called " sympathin ", the name originally coined by Cannon & Bacq (1931) to denote the adrenergic transmitter(s). This is done in spite of the fact that we are not certain whether the transmitter of sympathetic impulses really contains adrenaline in the percentage in which it occurs in nerves and ganglia. The term " sympathin " will also be used for the mixture of noradrenaline and adrenaline in the brain. It does not imply that the two substances serve the same function or always act jointly.

2. Estimation of Sympathin in Brain

The distribution of sympathin in the normal brain was determined on dogs bled under chloroform anaesthesia. Pieces of tissue weighing 10–250 mg. were rapidly dissected out, stripped of their pial cover and dropped into cold acidified ethanol (1 ml. conc. HCl/l. ethanol). They were ground up in a glass homogenizer, the homogenate was centrifuged, and the supernatant, to which washings of the tissue residue had been added, evaporated to dryness. This dry extract was purified by treating it with a succession of solvents (for details see Vogt, 1954), and the final extract applied to paper for chromatography in phenol–HCl (for procedure, and also for methods of elution and bio-assay, see Crawford & Outschoorn, 1950; Vogt, 1952). The purpose of the chromatography is, first, to separate adrenaline and noradrenaline from each other, as the bio-assay of mixtures is subject to large errors (Gaddum & Lembeck, 1949); secondly, to separate off other basic substances present in brain extracts and liable to interfere with the estimations. The regions to which the sympathomimetic amines had travelled were determined by spraying with ferricyanide a strip of the paper carrying markers of the pure substances; the corresponding parts of the paper along which the extracts had travelled were then eluted, the eluates evaporated in vacuo, and the residues dissolved in water. The noradrenaline was assayed on the blood pressure of rats sensitized by hexamethonium, and the adrenaline on the isolated rat uterus suspended at 30° C. in an aerated salt solution of low calcium content. Recovery experiments, checks for the identity of the compounds extracted, and precautions to avoid errors due to interfering substances are described in the original papers.

3. Distribution of Sympathin in Brain

It was found that traces of sympathin occurred in all parts of the dog's brain, as, indeed, they are found in all tissues of the body which contain postganglionic sympathetic fibres. There were, however, regions of the brain containing much more sympathin than others, and these differences were consistent in all individuals examined. The concentrations ranged from means as high as 1 μg./g. in the hypothalamus of the dog and 1.4 μg./g. in that of the cat to figures as low as 0.01 μg./g. in the dog's sensory roots or 0.04 μg./g. in its visual cortex. A glance at Table I, which contains the estimations of noradrenaline in a selection of the regions examined, shows that the concentrations do not follow the cellularity of the tissue: thus the very cellular cerebellar cortex and visual cortex (25, 20, Table I) contain only 7% and 4% of the sympathin found in the hypothalamus. Since the vascularity and, therefore, presumably, the vasomotor nerve supply, is largely

TABLE I. NORADRENALINE IN THE DOG'S BRAIN
(μg./g. fresh tissue)

	No. of dogs	Range	Mean
Concentrations above 0.24 μg./g.			
Diencephalon			
1. Hypothalamus (excluding 2 and 3)	31	0.6 –1.76	1.03
2. Corpora mammillaria	2	0.32–0.50	0.41
3. Regio preoptica	4	0.18–0.32	0.28
4. Medial thalamic nuclei	12	0.12–0.40	0.24
Mesencephalon			
5. Stratum griseum centrale	11	0.27–0.80	0.42
6. Mid-brain tegmentum	6	0.24–0.60	0.37
7. Red nucleus and fossa interpeduncularis	7	0.15–0.36	0.26
Medulla oblongata			
8. Region of nuclei X and XII	9	0.12–0.50	0.31
9. Formatio reticularis, posterior part	4	0.30–0.45	0.34
10. Formatio reticularis, anterior part	1	—	0.36
11. Area acustica	2	0.33–0.45	0.39
12. Central part of floor of fourth ventricle	5	0.10–0.40	0.27
13. Area postrema	13	0.70–2.00	1.04
Concentrations 0.2–0.1 μg./g.			
14. Motor cortical area **(4)***	5	0.10–0.24	0.18
15. Somaesthetic cortical area **(3)**	2	0.12–0.25	0.19
16. Superior colliculi	4	0.10–0.21	0.16
17. Inferior colliculi	4	0.10–0.16	0.11
18. Medial geniculate body	3	<0.09–0.15	0.13
19. Anterior horns	2	0.15–0.20	0.18
Concentrations below 0.1 μg./g.			
Grey matter			
20. Visual cortical area **(17)**	3	0.02–0.05	0.04
21. Acoustic cortical areas **(20** and **36a)**	4	0.04–0.06	0.05
22. Cingulate gyrus (all areas)	8	0.04–0.13	0.09
23. Lateral thalamic nuclei	7	0.03–0.12	0.08
24. Lateral geniculate body	2	0.06–0.08	0.07
25. Cerebellar cortex	4	0.01–0.10	0.07
26. Amygdaloid nuclei	3	0.03–0.10	0.06
27. Caudate nuclei	4	0.03–0.12	0.06
28. Tuber olfactorium	3	0.03–0.07	0.05
White matter			
29. Corpus callosum	1	—	0.08
30. Pyramid	1	—	0.06
31. Anterior roots	1	—	0.06
32. Optic nerve	1	—	0.02
33. Album centrale	1	—	0.02
34. Posterior roots	1	—	0.01
35. Cerebrospinal fluid	2	—	0.005

*** The bold numbers in brackets are those given by Klempin (1921) *J. Psychol. Neurol., Lpz.* 26, 229**

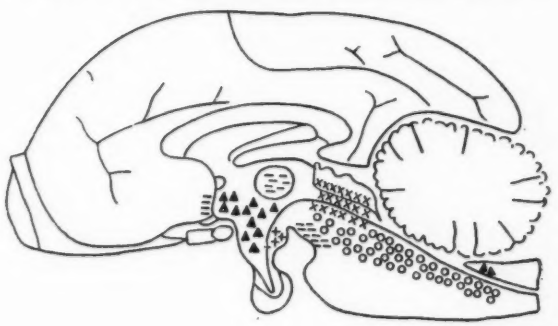
determined by the cell density, these low values in regions of high cellularity make it highly improbable that all brain sympathin is carried in vasomotor fibres. Indeed, the figures of 0.04–0.07 μg./g. supply a probable upper limit for the amount of sympathin contributed by vasomotor fibres in the most vascular parts of the brain. This point will be further discussed later.

Even where concentrations were low, differences appeared to be consistent: the motor and somaesthetic areas (14, 15, Table I) of the brain gave higher figures than the visual or acoustic areas (20, 21), the medial geniculate bodies (18) contained more than the lateral geniculate bodies (24), the medial thalamic nuclei (4) over three times as much as the lateral thalamic nuclei (23).

In fig. 1 the parts of the brain which are rich in sympathin are shown on a medial sagittal section. From the hypothalami, in which the concentration is highest, there is a decline caudally along the central part of the brain-stem; if we ascend from the hypothalamus, we find only the medial nuclei of the thalamus to contain a moderately high concentration, whereas the remaining diencephalon, and the whole of the telencephalon, including its basal ganglia, contain but little sympathin. Figure 1 further suggests that those regions which, according to Ranson & Magoun (1939), on electrical stimulation elicit generalized discharge in the peripheral sympathetic nerves, are rich in sympathin. The work on drugs to be described was carried out in an attempt to find whether this coincidence has any physiological significance.

A region with a high content of sympathin but not belonging to the central nervous system proper is the area postrema. This region has a great permeability to dyes, and it is perhaps no accident that other constituents of nervous tissue, like

FIG. 1. MEDIAL SAGITTAL SECTION OF A DOG'S BRAIN: DISTRIBUTION OF NORADRENALINE



Vogt (1954) by permission of Journal of Physiology

▲: 1.0 μg./g. X: >0.4<1.0 μg./g.
○: >0.3<0.4 μg./g. —: >0.2<0.3 μg./g. fresh tissue

5-hydroxytryptamine and substance P (von Euler & Gaddum, 1931), were also found concentrated in this small ridge of tissue (Amin, Crawford & Gaddum, 1954).

No information is available as to the histological nature of the structures containing the sympathin. Only one nervous element can be excluded, that is, the myelinated fibre. Without exception, all myelinated fibre tracts contain only traces of sympathin. Grey matter, as discussed before, may be either very poor or very rich in sympathin; if the sympathin is carried in neurones, it must therefore be localized either in cell bodies or in non-myelinated fibres or in both.

On estimating the noradrenaline concentrations in subdivisions of the hypothalamus, equal amounts were found in the anterior and the posterior parts; the dorsal half, however, contained less than the ventral half; and the lateral, less than the medial. Since the regions with less noradrenaline are those containing more myelinated fibres, these findings do not prove concentration gradients in the grey matter itself.

Though no detailed mapping was carried out in other species, the main features of the distribution were confirmed in the cat, and the hypothalamus of the rat was also found rich in sympathin.

The percentage of adrenaline in the sympathin of the dog's brain (and of the dog's cerebrospinal fluid) varied from 6% to 25% (mean, 13.7%); this is a higher percentage than that for sympathetic ganglia, which varied from 0.8% to 7.0%, with a mean of 2.4%. The percentage of adrenaline in the sympathin of the cat's brain was only about one-half of that found in the dog.

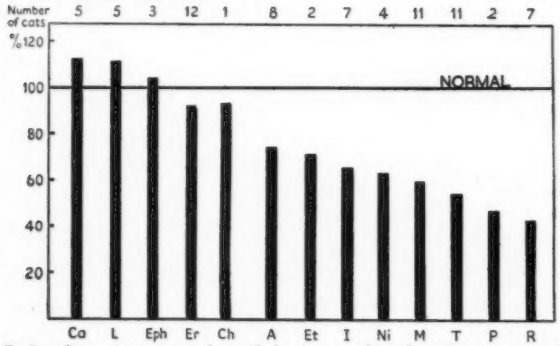
4. Action of Drugs on Sympathin in Brain

The object of these experiments was to see whether changes in the functional state of the brain as they may be produced by drugs acting on the central nervous system would be reflected by changes in the distribution pattern of sympathin. The drugs were usually injected subcutaneously and allowed to act for a period of 4–5 hours, as preliminary experiments had shown that shorter duration did not produce any changes.

The first region to be investigated was the hypothalamus. The results obtained on cats with 13 different drugs are summarized in fig. 2 and fall into two groups. The five drugs shown on the left side of the figure did not cause significant changes in the noradrenaline concentration, whereas the eight drugs on the right side of the figure caused falls of varying severity. Rises outside the normal range were never observed.

It was stated earlier that, in the normal brain, noradrenaline is accompanied by a small amount of adrenaline, ranging in cats from 2.5% to 11% of the total amines. Experiments were carried out to see whether drugs produced a change in the ratio of the two amines. Of the five drugs used, three (β-tetrahydronaphthylamine, morphine and picrotoxin) were among those found to lower the noradrenaline content of the hypothalamus, and two (caffeine and ergometrine) belonged to those found inactive in that respect. The percentage of adrenaline in the hypothalamic sympathin was found to be

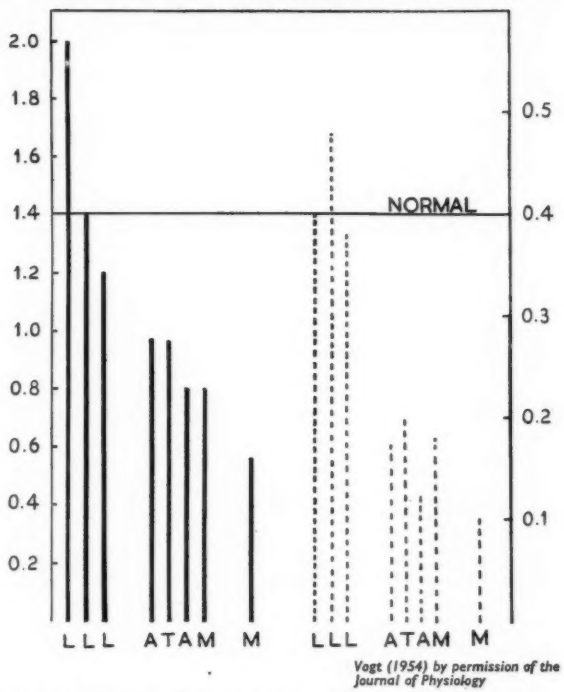
FIG. 2. EFFECT OF 13 DIFFERENT DRUGS ON NORADRENALINE CONTENT OF HYPOTHALAMUS IN GROUPS OF CATS



Each column represents hypothalamic noradrenaline in a group of cats, following the administration of one drug. The figures for the mean concentration are expressed as a percentage of the mean of 1.4 μg./g. found in 20 untreated cats.

Ca: caffeine (90–292 mg./kg.); L: leptazol (50–60 mg./kg.); Eph: ephedrine sulphate (35–50 mg./kg.); Er: ergometrine maleate (3.5–20 mg./kg.); Ch: chlorpromazine HCl (25 mg./kg.); A: apomorphine HCl (75–122 mg./kg.); Et: ether (anaesthetic dose); I: insulin (1.5–1.8 i.u./kg.); Ni: nicotine bitartrate (12–17 mg./kg.); M: morphine HCl (30–60 mg./kg.); T: β-tetrahydronaphthylamine (40–60 mg./kg.); P: picrotoxin (1.0–1.5 mg./kg.); R: reserpine (0.4 mg./kg.)

FIG. 3. HYPOTHALAMIC NORADRENALINE AND MESENCEPHALIC NORADRENALINE OF 8 CATS



Vogt (1954) by permission of the Journal of Physiology

Left ordinates: hypothalamic noradrenaline (μg./g. fresh tissue)

Right ordinates: mesencephalic noradrenaline (μg./g. fresh tissue)

Each solid line: hypothalamic noradrenaline of one cat

Each interrupted line: corresponding figure for mesencephalic noradrenaline

Drugs and doses as in fig. 2.

within the normal range in all 22 experiments. When, therefore, as a result of drug action, noradrenaline is lost from the hypothalamus, adrenaline is also lost and the proportion in which they occur in the tissue remains the same.

The concentration of noradrenaline was estimated not only in the hypothalamus, but also in the tegmentum of the midbrain of cats, as illustrated in fig. 3. It is obvious that drugs lowering the level of the hypothalamic noradrenaline also lowered its mesencephalic component and that drugs without effect on the one were without effect on the other.

The high concentration of noradrenaline in the area postrema prompted the investigation of its response to the administration of drugs. The experiments were carried out on dogs, since estimation of noradrenaline in the area postrema of cats is made difficult by the presence of substances interfering with the assays. Ether (anaesthetic dose), morphine (53–60 mg./kg.) and apomorphine (43–50 mg./kg.) were used. The first two drugs caused a fall in the noradrenaline content of the hypothalamus, whereas apomorphine had no such effect; none of the drugs, however, produced any change in the noradrenaline content of the area postrema. The area postrema is considered to contain chemo-receptors for the vomiting centre (Wang & Borison, 1950; Borison & Brizzee,

1951), and the fact that apomorphine did not cause any change in its noradrenaline content is noteworthy, but its interpretation uncertain. It may mean that sympathin does not take part in the physiological events leading to the stimulation of chemo-receptors in the area postrema by apomorphine; it may also mean that such stimulation is very transient—as is the emesis—and that therefore the stores of sympathin would not be lowered even if sympathin were implicated in this particular activity.

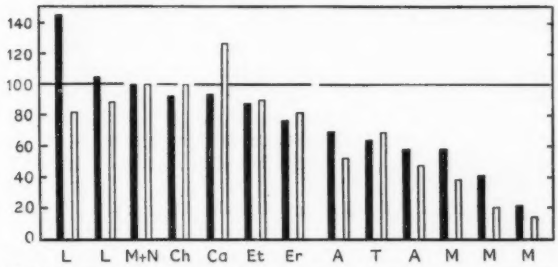
5. Comparison of the Effects of Drugs on Sympathin Content of the Hypothalamus and on Activity of the Sympathetic Centres

The eight " active " drugs shown in fig. 2 produce the most diverse central effects: convulsions, anaesthesia, tranquillization or autonomic effects may be prominent; convulsive and tranquillizing drugs are also represented among the " inactive " drugs on the left of the picture, so that these properties are obviously not decisive. An action common, however, to at least five of the eight " active " drugs is a stimulation of the sympathetic centres: this is known for ether, insulin, nicotine, morphine and β-tetrahydronaphthylamine; if there were a correlation between this effect of the drugs and the depletion of hypothalamic noradrenaline, the other three drugs shown on the right in fig. 2—apomorphine, picrotoxin and reserpine —should also cause central sympathetic stimulation, whereas the drugs on the left should lack such an action.

This theory was put to the test by experiments in which the discharge of adrenaline and noradrenaline from the adrenal medulla was used as a convenient indicator of central sympathetic activity. (Release of adrenaline through direct action of drugs on the adrenal medulla is rare; of the drugs in question, only nicotine is known to have this effect.) A first experiment was carried out on rats: it showed that picrotoxin, one of the drugs which should have a stimulating effect on sympathetic centres, did in fact cause a discharge of adrenaline from the adrenal medulla, just as did morphine, whereas ergometrine, which is inactive on the cat's brain, had no such effect. Then the work was continued on cats, in which release of amines from the adrenal medulla and concentration of hypothalamic noradrenaline could be measured on the same animal. Because of the great variation in the amounts of hormones stored in the adrenal medulla of different animals, the drug was allowed to act on one adrenal only, whereas the contralateral gland of the same cat was used as control. This was done by carrying out a unilateral adrenal denervation between two and three weeks before the experiment. This procedure shields the denervated gland from the central secretory impulses elicited by the drug, and the loss of amines from the normal (innervated) gland in relation to the content of the resting (denervated) gland serves as a measure of central sympathetic stimulation. The difference between the amine content of the innervated and denervated adrenal of normal cats not treated with drugs (and not subjected to natural excitement) is very small and does not exceed 10%.

Figure 4 shows the results of experiments in which drugs were administered to cats as before and estimations made of the noradrenaline content of the hypothalamus and hormone content of the two adrenals (adrenaline and noradrenaline were measured separately and then added together). It is clear from fig. 4 that only those cats showed a significant fall in hypothalamic noradrenaline which also lost a large proportion of amines from the innervated gland. It is further confirmed that leptazol, caffeine and chlorpromazine affect neither the concentration of hypothalamic sympathin, nor that of the adrenomedullary hormones, whereas apomorphine, one of the drugs about which information had been missing, and which was suspected to cause central sympathetic stimulation, is indeed shown to possess this effect.

FIG. 4. EFFECT OF DIFFERENT DRUGS ON NORADRENALINE CONTENT OF HYPOTHALAMUS AND HORMONE CONTENT OF THE ADRENAL MEDULLA OF INDIVIDUAL CATS



Black columns: hypothalamic noradrenaline, expressed as percentage of mean of 20 untreated cats

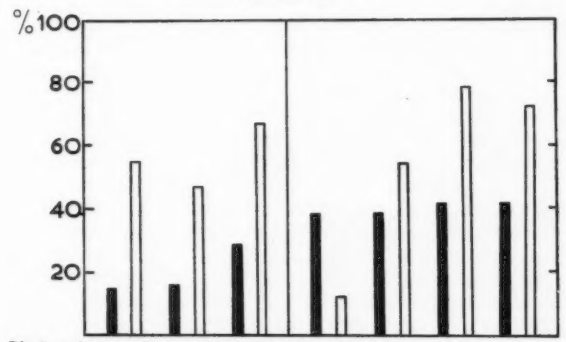
White columns: total amines of the innervated adrenal medulla of the same cats, expressed as percentage of the amines in the contralateral denervated medulla

Doses and drugs as in fig. 2.

The combination M + N was morphine (40 mg./kg.) and nalorphine (37.5 mg./kg.).

Last, reserpine was examined with regard to its effect on sympathetic centres (Holzbauer & Vogt, 1956). The results in two litters of cats are represented in fig. 5. From estimations on the first litter, it is obvious that there was amine

FIG. 5. THE EFFECT OF RESERPINE ON TWO LITTERS OF CATS



Black columns: hypothalamic noradrenaline

White columns: total amines of the innervated adrenal medulla, expressed as percentage of the amines in the contralateral denervated medulla

The animals in each half of the figure are litter mates.

Left part of figure: all cats given 0.4 mg./kg. reserpine intraperitoneally the day before the experiment. Hypothalamic noradrenaline is shown as percentage of mean of 20 controls.

Right part of figure: 3 cats given 0.4 mg./kg. reserpine as above; one cat given 0.7 mg./kg. Hypothalamic noradrenaline is shown as percentage of amount determined in untreated control from the same litter.

depletion of the hypothalamus and of the innervated medulla. In the second litter—only four cats were given injections, the fifth cat serving as control—loss of noradrenaline from the hypothalamus was also severe, the concentrations being almost the same in the four cats, but the decrease in medullary amines from the innervated glands, though present, was very variable. This finding suggests that the action of reserpine is more complex than that of the drugs hitherto discussed, and is not strictly related to a stimulation of the sympathetic centres. In view of the fact that reserpine renders tissues—the brain included—incapable of retaining another base, 5-hydroxytryptamine (Pletscher, Shore & Brodie, 1956; Paasonen & Vogt, 1956), it might also interfere with the retention of noradrenaline in certain tissues. However, 0.4 mg./kg. of reserpine does not appear to liberate any amines from the denervated adrenal medulla, since the range of concentrations (0.5–1.1 mg./g. adrenal) found in the seven cats given reserpine was precisely the same as that observed in a group of cats which did not receive reserpine. On the other hand, recent experiments (Muscholl & Vogt, 1957) have shown that reserpine produces a loss of noradrenaline from sympathetic ganglia of the rabbit, a single injection of 1 mg./kg. or more causing about 80% of the amine to disappear. Response to electrical stimulation of preganglionic and postganglionic sympathetic fibres is impaired under these conditions, and this may contribute to the signs of miosis, relaxation of the nictitating membrane and ptosis seen to follow injections of reserpine. Activation of the sympathetic centres would thus no longer be manifested by peripheral signs mediated through adrenergic nerves; medullary secretion, on the other hand, which is mediated by cholinergic neurones, can take place as long as the stores of medullary amines are intact. In the experiments illustrated by fig. 5, the loss of amines from the innervated adrenal medulla constitutes a proof of central sympathetic activity following the injection of the drug. Its lack of correlation with the fall in the noradrenaline content of the hypothalamus suggests that the effect on the hypothalamus is essentially in the nature of an interference with the storage of the noradrenaline rather than a result of increased central activity; what central sympathetic activation is seen after reserpine may, in fact, be a compensatory phenomenon rather than a direct effect of the drug.

A few examples of combined drug action furnish another opportunity of testing the relation between stimulation of sympathetic centres and effect on the noradrenaline content of the hypothalamus. When nalorphine was used to suppress the signs of excitation by morphine, there was no loss in hypothalamic noradrenaline or in medullary amines (fig. 4, column 3). When, however, morphine was combined with chlorpromazine (25 mg./kg., Holzbauer & Vogt, 1954), loss in hypothalamic noradrenaline and secretion of amines from the innervated adrenal were just as large as with morphine alone. Thus chlorpromazine, in spite of its "tranquillizing" effect, does not antagonize the sympathetic activity produced by morphine, or its effects on hypothalamic noradrenaline and medullary amines. Chlorpromazine (fig. 2, column 5 and fig. 4, column 4) has no effects of its own on the concentration of sympathomimetic amines.

In view of the fact that loss of noradrenaline from different parts of the brain was always accompanied by an increase in secretion from the adrenal medulla, the question has been asked whether a causal relation might exist between increase in circulating medullary hormones and loss of sympathin from the brain. Such a possibility was eliminated by carrying out experiments in which morphine was administered to cats that had been previously subjected to bilateral adrenal denervation. The fall in hypothalamic noradrenaline in these animals was as large as in cats with intact adrenal nerves, in spite of the fact that medullary amines were not circulating in the blood of these animals.

6. Conclusions

As stated earlier, the normal pattern of distribution of sympathin in the brain makes it very unlikely that, in those parts where the concentration is high, we are dealing with substances entirely situated in vasomotor nerves. This is particularly true in view of the fact that most of the vasomotor nerves are found in the pia mater (Pfeifer, 1930), which is dissected off before extraction of the tissue. This conclusion is further supported by the following experiment: when the superior cervical ganglia are extirpated in cats, and time is allowed for the degeneration of the postganglionic sympathetic fibres, the hypothalamic noradrenaline concentration remains unchanged. Finally, the observation, that drugs lower the sympathin content of brain only when they also stimulate the sympathetic centres, appears to relate the action of drugs to the neuronal activity of the regions and not to a vasoconstriction for which there is no evidence, and which is unlikely to occur in an active tissue.

Concerning those regions of the brain which, like the cerebellar or the cerebral cortex, contain little sympathin in spite of being highly vascularized, it is impossible to decide whether their sympathin is made up entirely of transmitter carried by vasomotor fibres or is of more complex origin.

The evidence reviewed in this paper points to a part played by sympathin in the activity of the regions in which it is concentrated, but it gives us no lead concerning the actual role these amines may play in nervous activity.

In peripheral adrenergic nerves, sympathin acts as a transmitter; at synapses, it modifies cholinergic transmission. It is possible that one of these roles—or both—is also played by diencephalic sympathin. If it were a transmitter, one would have to assume that it is localized in neurones. Even on that point we have no evidence: it might just as well be carried in the glia, and has indeed been shown to occur in certain gliomas (Bülbring, Philpot & Bosanquet, 1953). The known transmitters of peripheral nerves are present in the entire neurone, the whole axon included. If sympathin were a central transmitter, the only neurones for which it could carry out transmitter function would be those with non-myelinated axons, since myelinated fibres were consistently found to be lacking in sympathin.

Adrenaline has also been assigned a neurosecretory role in transmitting stimuli from the hypothalamus via the portal vessels to the anterior lobe of the pituitary, thus causing the release of gonadotrophins (Sawyer, Markee & Everett, 1950). The region responsible for causing the release of gonadotrophins is either identical with, or very near, the median eminence. This region is apparently not singled out by a particularly high concentration of sympathin. Thus, although the view that some of the hypothalamic sympathin is transmitting impulses to the pituitary is compatible with the facts relating to the distribution of sympathin, it is not specifically supported by them.

Another central function of the sympathomimetic amines

has been discovered by Bonvallet, Dell & Hiebel (1954), who showed that they are potent stimulants of the ascending activating system of the reticular formation. One may therefore wonder whether the sympathin found in these regions has a share in some function specific for these parts of the reticular formation. Any hypothesis conceived on those lines is hampered by our ignorance about the precise anatomical relationship between the reticular formation and the sympathetic centres and pathways.

ACKNOWLEDGEMENT

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THE ACTION OF HUMORAL TRANSMITTERS ON SMOOTH MUSCLE

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1 Innervation
2 Peristaltic reflex
3 Tonus
4 Oxygen consumption
5 Intracellular electrical recording
6 Stretch
7 Auto-rhythmicity
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10 Potassium flux
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1. Innervation

This account of the action of humoral transmitters on smooth muscle is based entirely on observations made on one particular type, the intestinal muscle, which has a double, antagonistic, cholinergic and adrenergic innervation, and which, of all the different smooth muscle types in the body, has been most thoroughly studied. Paton (1955, 1956) has introduced a method for coaxial electrical stimulation of isolated loops of intestine by which a twitch can be elicited due to the excitation of the intramural nerve fibres. Pharmacological evidence, and the fact that acetylcholine is the only substance the release of which significantly increases during stimulation, indicate that acetylcholine is the transmitter liberated at the postganglionic motor endings. The presence of inhibitory neurones in the wall of the stomach (Paton & Vane, 1956) and of the intestine (Ambache, 1951) has also been demonstrated, and all the evidence supports the assumption that the transmitter substance is adrenaline, or noradrenaline, or a mixture of both.

2. Peristaltic Reflex

In addition to an extrinsic innervation, the gut also possesses an intrinsic nervous plexus. The latter is known to be chiefly concerned with the peristaltic reflex, which consists of coordinated muscle contractions in response to distension leading to the propulsion of the intestinal contents. None of the extrinsic nerves plays any role in this myenteric reflex, as has recently been shown once more by chronic denervation experiments allowing nerve degeneration (Bülbring, Lin & Schofield, unpublished). The site of the sensory receptors registering the distension appears to be in the mucosal layer, since the reflex is abolished by removal of the mucous membrane. Many ganglion cells in the submucosal plexus of Meissner are not unlike the cells of the dorsal root ganglia; they are unipolar cells whose axon divides into two processes running in opposite directions, one towards the mucous membrane and the other towards Auerbach's plexus. One may assume, therefore, that Meissner's plexus is mainly sensory, while Auerbach's plexus is mainly motor. The ganglion cells of the latter are monopolar, bipolar, or multipolar, sending many small branches to the smooth muscle fibres which are thus innervated individually (Schofield, 1957).

3. Tonus

Though ganglion-blocking agents (e.g. hexamethonium) abolish the peristaltic reflex, and though atropine abolishes the effect of excitation of the postganglionic structures, it is well known that in the presence of both these substances the intestine still exhibits pendular activity and maintains a tone. This property is also preserved in nerve-free and ganglion-free preparations of intestinal muscle which are still able to respond to stretch by increasing their spontaneous activity and tone (Evans & Schild, 1953).

Tonus of smooth muscle has been defined by Lovatt Evans (1926) as the muscle's resistance to extension. Bozler (1948) described tonus as a fluctuating level of tension or length on which brief contractions were superimposed. He assumed that spontaneous fluctuations of the muscle's metabolism led to changes in electrical potential which he observed to be associated with the changes in tension.

4. Oxygen Consumption

The puzzling observation that smooth muscle could apparently contract without increasing its oxygen uptake has more recently been elucidated by comparative measurements of oxygen consumption in isotonic and isometric conditions (Bülbring, 1953). A polarographic method (Davies & Brink, 1942) was used for recording the oxygen uptake continuously and simultaneously with the muscle's mechanical activity. The experiments were carried out on the taenia coli of the guinea-pig, a narrow band of parallel fibres constituting the longitudinal layer of the caecum. It was found that the rate of oxygen consumption was influenced (i) by the length of the muscle, and (ii) by the tension produced by the muscle resisting the extending force of the lever. Thus in isotonic conditions the metabolic effect of a relaxing agent, e.g. adrenaline, depended on the weight of the lever: if the lever was light, the lengthening of the muscle during the adrenaline relaxation was accompanied by an increased oxygen consumption. If the lever was weighted—which also increased the fibre length and the oxygen uptake—and if then the muscle was relaxed by adrenaline, momentarily abolishing the muscle's resistance to extension by the heavy lever, the oxygen consumption fell in spite of further lengthening of the fibres. Conversely, acetylcholine applied to a muscle attached to a light isotonic lever reduced oxygen consumption, since the fibre length was reduced as the muscle shortened against a small weight. When, however, the acetylcholine contraction took place against a heavily weighted lever, oxygen uptake increased in spite of the shorter fibre length. As a consequence of the two opposing factors operating in isotonic conditions, the absolute changes in oxygen consumption are much smaller than in isometric conditions. Here the fibre length is constant. The muscle is, however, not at rest; some degree of tension or tone is maintained by the muscle, which varies in different preparations; and no true resting oxygen consumption can be deter-

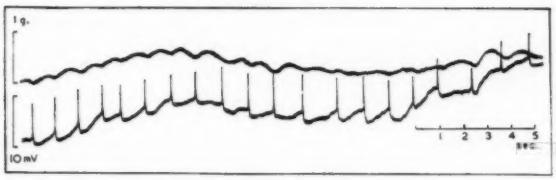
mined in the continuously active tissue. Nevertheless, the rate of oxygen uptake is, over a wide range, proportional to the tension which the muscle produces. Not only is an increase in tension in response to acetylcholine accompanied by an increase in oxygen consumption proportional to activity, but also the much smaller spontaneous fluctuations of the muscle tone are associated with parallel fluctuations in oxygen uptake. By measuring the heat production in isometric conditions, Bozler (1930) had already found that the maintenance of tension by smooth muscle required a continuous energy expenditure which differed only quantitatively from that observed in striated muscle. The mean oxygen consumption by taenia coli measured during its basic spontaneous activity is only about one-quarter of that found in striated muscle during rest.

5. Intracellular Electrical Recording

Electrical records from the smooth muscle of the taenia coli, using intracellular electrodes, have helped in understanding its behaviour. This tissue normally undergoes large changes in length and tension, being situated in the wall of a hollow organ of variable inside pressure and volume and, in addition, it changes its tension spontaneously in a continuous rhythm known as pendular activity.

The membrane potential of the smooth muscle is neither so high nor so stable as that of striated muscle. The average value is 50 mv and the scatter is very large, ranging from 25 to 75 mv (Bülbring & Hooton, 1953; Greven, 1953; Bülbring, 1954; Woodbury & McIntyre, 1954). The potential fluctuates spontaneously and there is a continuous rhythmic discharge of spikes at an average rate of one per sec. (fig. 1). Thus no true resting potential can be determined because the cells are in a state of continuous activity, and the wide scatter of the potential values recorded is probably partly due to the variable degree of activity of the cells at the moment of impalement.

FIG. 1. SPONTANEOUS RHYTHMIC ACTIVITY OF TAENIA COLI



Upper record: tension (g.)
Lower record: membrane potential (mv)
Note small change in tension following each spike.

6. Stretch

During attempts to fix strips of taenia as rigidly as possible for intracellular electrical recording it was noticed that the height of the membrane potential depends on the muscle length; the more the muscle is stretched, the lower is the membrane potential (Bülbring, 1954, 1955). When the muscle is stretched, the frequency of spike discharge also increases (Table I). The mechanism of this reaction is probably similar to that observed in muscle spindles (Katz, 1950). The deformation of the cell leads presumably to depolarization which

TABLE I. THE EFFECT OF STRETCH RECORDED IN A TYPICAL EXPERIMENT

Length (mm.)	Tension (g.)	Membrane potential (mV)	Spike frequency/min.
8	3.2	45	68
9	7.5	40	88
10	10.2	36	120

The experiment was carried out in the presence of atropine 5×10^{-9}.

initiates or facilitates the discharge of impulses. As the frequency of spike discharge increases, the muscle tension rises. Experiments in which the muscle was stretched to a given length and subsequently released, when it reassumed its former length but produced a different tension, revealed that the membrane potential and the spike frequency are only indirectly related to the muscle length, but that they bear a close correlation with the tension which the muscle produces as a result of its length being varied. The smooth muscle cell thus combines properties of a sensory organ, i.e. a stretch receptor, with a contractile system (Bülbring, 1955).

7. Auto-Rhythmicity

The spike potentials which smooth muscle produces spontaneously are smaller than the action potentials of normal striated muscle, of longer duration and of different shape. However, if striated muscle is deprived of calcium it also becomes rhythmically active and the instability of the membrane which leads to the spontaneous discharge of impulses confers upon it the ability to react like a sensory organ, i.e., to respond to mechanical or chemical stimuli by increasing the frequency of its discharge (Adrian & Gelfan, 1933; Bülbring, Holman & Lüllmann, 1956). The configuration of the action potentials which calcium-deficient striated muscle discharges spontaneously bears certain similarities to those of normal smooth muscle. One characteristic in particular, i.e., a period of slow depolarization leading up to the spike deflection, a " prepotential ", is seen in all tissues possessing auto-rhythmicity. It is regularly observed in smooth muscle, where it is variable in degree and apparently dependent on the potential level. The configuration of the spike potentials also varies spontaneously and is in a similar way dependent on the height of the membrane potential.

8. Correlation between Membrane Potential, Spike Frequency, Spike Configuration and Tension

There are two main types of slow waves of membrane potential occurring spontaneously. The first consists of fluctuations recurring at a frequency of about one per sec.; these are the prepotentials and usually carry the spike. The second type of slow wave is much slower and the full cycle lasts on an average about two minutes. These waves are associated with the pendular activity and they are related to changes in the frequency of spike discharge.

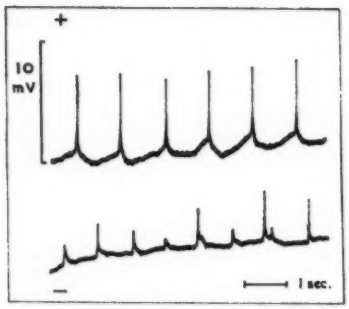
The height of the spikes may vary from a few millivolts to 60 mv. Usually the spike causes only partial depolarization, but the spike may reach zero or overshoot by a few millivolts (Bülbring & Holman, unpublished observations; see also Woodbury & McIntyre, 1956). The duration of the spike

ranges from 20 to 100 msec. The rising phase may be longer than the falling phase, or vice versa, or the two phases may be of equal duration. In spite of these very wide variations, a correlation between the size and shape of the spikes on the one hand, and the membrane potential on the other hand, has been established (Bülbring, 1956a, 1957).

The state of polarization of the membrane determines the rate of discharge. It is likely that potential changes spread across sites of low resistance from cell to cell (Prosser, unpublished communication to XX° Congrès International de Physiologie, Bruxelles, 1956), thus synchronizing the activity of large numbers of cells, since each spike is followed by a small development of tension. Whether the net tension produced by a few millimetres of muscle increases or falls depends therefore on whether the individual increments in tension occur sufficiently frequently to summate or not.

Spontaneous changes of membrane polarization and alterations caused by varying the muscle length have already been mentioned. More uniform potential changes can be produced by applying polarizing currents or pharmacological agents. It has been found that the effects of the two antagonistic transmitter substances are very similar to those of electrotonic currents of opposite polarity (fig. 2). During

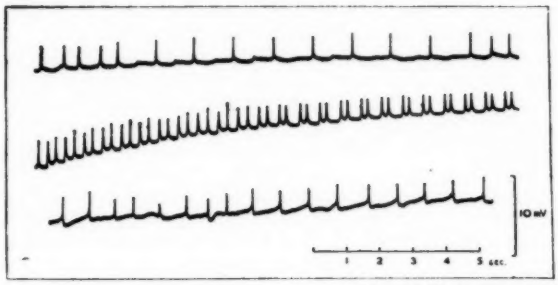
FIG. 2. CHANGE IN SPIKE FREQUENCY AND CONFIGURATION PRODUCED BY ELECTROTONIC CURRENTS



Upper record: effect of anodal current (+)
Lower record: effect of cathodal current (−)

depolarization (e.g., by acetylcholine or by cathodic polarization) the frequency of spike discharge is accelerated. The individual spike duration is prolonged chiefly because of a slower rate of repolarization, and the next spike may arise before repolarization of the previous spike is complete, which leads to the discharge of double spikes (fig. 3). At the same time the small increments of tension which follow each spike summate and the net tension builds up. On the other hand a rise in membrane potential (caused, for example, by adrenaline or by anodic polarization) slows the rate of spike discharge. The spike duration is shortened and there follows a prolonged phase of hyperpolarization (fig. 4). The recovery from the hyperpolarization following each spike merges with the slow depolarization leading up to the next spike but, as the prepotential now has to proceed over a longer time before the firing level is reached, the intervals between spikes are increased. For this reason the tension cannot build up and the muscle relaxes. This mechanism by which the state of

FIG. 3. EFFECT OF ACETYLCHOLINE ON DISCHARGE OF SPIKE POTENTIALS



Bülbring (1956b) by permission of Gastroenterologia

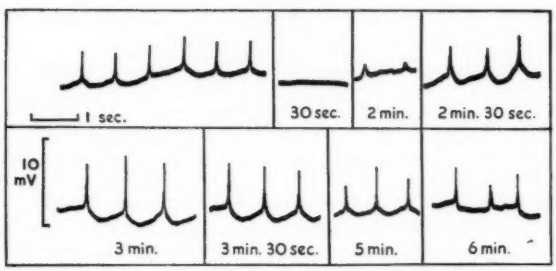
Top record: normal spike discharge
Middle record: acceleration and double spikes during the effect of 5 μg. of acetylcholine
Lower record: taken as the effect passed off

polarization determines the spacing of the spikes and thus, of necessity, the level of tension, provides the explanation for the close correlation which exists between the three variables.

9. Effect of Reducing the Sodium-Ion Concentration

Recent observations (Holman, 1957), in which the influence of changing the NaCl concentration has been studied, have shown that the spontaneous electrical activity of taenia coli was not altered when all the NaCl was removed from the surrounding fluid and replaced by choline chloride. The production of spike potentials continued at a normal frequency and there was no change in height or shape of the spikes. Only complete absence of all sodium ions gradually brought the spike discharge to a stand-still. One may conclude that the spike potentials are produced in smooth muscle by a mechanism qualitatively similar to that existing in other excitable tissues, since the presence of sodium ions is necessary. On the other hand, the carrier mechanism may be different in smooth muscle, since spikes can arise at such extremely low sodium concentrations, at which for example the pace-maker activity of heart muscle ceases.

FIG. 4. EFFECT OF ADRENALINE ON DISCHARGE OF SPIKE POTENTIALS



First record: discharge before injection of adrenaline (5 μg.)
Following records: discharge at specified times (30 sec. to 6 min.) after injection

10. Potassium Flux

Measurements of the movement of potassium using ^{42}K have shown that the K-flux in the guinea-pig's intestinal smooth muscle (Born & Bülbring, unpublished observations) is slower than that in mammalian striated muscle of the rat's diaphragm (Creese, 1954). Though the K-exchange of striated muscle has been measured in the resting state it is 10 times greater than that of the smooth muscle in its basic state of activity. The reason for this is the difference in cell size. The diameter of the smooth muscle cell is only one-fifth of that of a striated muscle fibre. Consequently, the total surface area of the smooth muscle cells is considerably greater. During its spontaneous rhythm, changes in membrane permeability recur at frequent intervals; periods of increased loss of K coinciding with greater tension alternate with periods in which the K-loss diminishes as the muscle relaxes. Whenever the muscle produces a tension, for example, in response to acetylcholine, this is associated with a greater rate of loss of K, while the relaxation which follows—or the relaxation produced by adrenaline—is associated with a greater rate of uptake of K (Born & Bülbring, 1956).

It is not known how far the shape of the spike potentials is influenced by processes concerned with active ion transport. The observation, however, that in nerve the phase of repolarization has a similar temperature coefficient to that of the sodium-pump mechanism has led to the belief (see Stämpfli, 1956) that enzymic processes are involved. This view is supported by observations on smooth muscle: in those conditions in which the rate of repolarization is slowed, for example during the action of acetylcholine, the rate of potassium influx is reduced, while K-uptake is greater in those conditions in which repolarization is fast and the spike is followed by a prolonged phase of hyperpolarization. These results suggest that the duration of the falling phase of the spike may be related to the activity of the sodium-pump.

11. Metabolic Inhibitors

This view is further supported by experiments in which the action of metabolic inhibitors was investigated, especially that of 2 : 4-dinitrophenol (DNP). DNP increases the rate of loss of K and diminishes the rate of K-uptake in smooth muscle (Born, Bülbring & Lüllmann, unpublished observation). As its first effect DNP causes a rise in spike frequency, and the spike duration becomes progressively longer; in the presence of DNP the phase of repolarization may last several hundred milliseconds, and spikes with a rapid falling phase are never seen. In spite of the membrane excitation, however, the muscle tension falls. Acetylcholine is still able, in the presence of DNP, to cause the usual membrane excitation but it fails to cause an increase in tension. Thus DNP abolishes the normal correlation between the events at the membrane and the contractile mechanism (Bülbring & Lüllmann, 1957). At this stage of the DNP poisoning the adenosinetriphosphate (ATP) concentration of the tissue is scarcely reduced (Born & Bülbring, 1955; Born, 1955, 1956); this indicates that the disruption of the chain of reactions coupling the membrane with the contractile system can occur although the diminution of the ATP concentration is only very small. It is interesting to note that the effect of DNP on the rate of oxygen consumption coincides in magnitude and in its time-course with its effect on membrane activity.

12. Summary

The smooth muscle of the longitudinal layer of the intestine possesses auto-rhythmicity and behaves like a continuously discharging sensory organ. It responds to mechanical or electrical or chemical stimuli by changing the frequency of its spontaneous spike discharge. It registers changes in muscle length and alters its spike frequency and tension accordingly. Since each spike is followed by a small production of tension, the spacing of the spikes determines whether the individual changes summate or not, i.e., whether the tension builds up or declines. Thus any mechanical change observed depends on a modification of the spontaneous electrical activity. Humoral transmitters, like electrotonic currents, act by causing a change in the state of polarization of the cell on which the frequency of spike discharge depends. Excitation and inhibition, contraction and relaxation, are not two different mechanisms; they are two different degrees of activity, either more or less than the initial activity, and they are achieved by diminished or increased polarization of the membrane. Biochemical processes concerned with ion transport appear to influence the time-course of the electrical manifestations, and they also seem to form part of the chain of reactions which links the events at the membrane with the mechanical system.

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MODE OF ACTION OF AUTONOMIC TRANSMITTERS ON THE HEART

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Physiologists have long been attracted to study the autonomic innervation of the heart both for its own sake and in the hope of gaining knowledge about the mechanism of excitation and inhibition at synapses. From the latter point of view the heart offers great facilities: it is accessible and comparatively robust; it may be isolated, perfused, drugged and subjected to changes in the ionic environment; the vagal and sympathetic nerve supply may be left intact and prepared for stimulation at will. Furthermore, the identities of both the excitatory and the inhibitory chemical transmitter have been established, so that nerve stimulation may be imitated by the application of adrenaline and acetylcholine. With the introduction of the intracellular microelectrode and of radioactive isotopes the inherent advantages of the heart have once more been turned to account, and some recent experiments with these tools will here be reviewed. It will be helpful to start by briefly describing the electrical activity of cardiac muscle in terms of the ionic theory (Hodgkin, 1950, 1951; Weidmann, 1956). A short account of the characteristic behaviour of the pace-maker fibres of the heart is also given, since these fibres seem especially sensitive to the action of the autonomic transmitters.

1. Electrical Properties of Cardiac Muscle

The absolute value of the membrane potential and the magnitude and time-course of the action potential have been recorded from Purkinje's fibres of the mammalian heart (Coraboeuf & Weidmann, 1949; Draper & Weidmann, 1951) and from ventricle and auricle fibres of vertebrate hearts (Woodbury, Woodbury & Hecht, 1950; Trautwein & Zink, 1952; Hoffman & Suckling, 1952; Burgen & Terroux, 1953a). In most cases a steady membrane potential of 60–90 mv (inside of the cell negative) has been found during diastole. As in other excitable tissues, this resting potential may be attributed to (i) the unequal distribution of different ions between the interior of the cell and the exterior, (ii) the selective permeability of the membrane. Thus inside the fibres potassium is present in high concentration compared with the outside, while sodium and chloride are present in low concentrations (for references see Weidmann, 1956). If the membrane were permeable to potassium alone, the potential difference necessary to balance the tendency of potassium to leave the cell—i.e., the equilibrium potential (E_K)—would be given at 20° C. by

$$E_K = 58 \text{ mv} \log \frac{(K_i)}{(K_o)}$$

where (K_i) and (K_o) are the activities of potassium ions in the fibre water and in the external solution. If the membrane were selectively permeable to chloride, the potential required to oppose the entry of chloride into the cell would be given by

$$E_{Cl} = 58 \text{ mv} \log \frac{(Cl_o)}{(Cl_i)}$$

Both the potassium and the chloride equilibrium potential have the same sign as the experimentally found resting potential; the resting potential may therefore be maintained by both these ions, the contribution of each depending on the concentration ratio and the permeability. The equilibrium potential for sodium ions is given by

$$E_{Na} = 58 \text{ mv} \log \frac{(Na_i)}{(Na_o)}$$

and has the opposite sign to that of the resting potential. Even a low permeability to sodium will therefore tend to depolarize the membrane and lead to a loss of cellular potassium and a gain of chloride, unless the difference between the concentration of these ions inside and outside the cell is maintained by a process consuming metabolic energy.

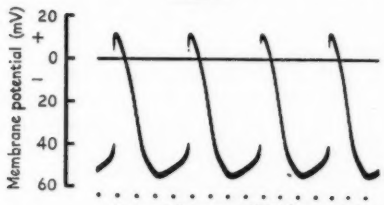
The amplitude of the cardiac action potential is greater than the resting potential: that is, the potential across the membrane at the beginning of systole has the opposite sign to that of the membrane at rest. In the giant axon of the squid, where the nature of the action potential is best understood, the reversal of the membrane potential during activity is explained by a sudden increase in the sodium permeability to a value much higher than that of the permeability to potassium and chloride (Hodgkin & Katz, 1949). In the heart, evidence for an increase in sodium permeability on excitation has so far been obtained only in the case of Purkinje's fibres (Draper & Weidmann, 1951). But it may reasonably be supposed that the action potential in myocardial fibres arises from a similar change in permeability.

A characteristic feature of cardiac muscle is the long duration of the action potential (Burdon-Sanderson & Page, 1883). Possible reasons for this peculiarity are best discussed after considering the factors determining the time-course of the action potential in squid axon. There the action potential is of short duration because (i) the process responsible for the rise in sodium permeability is rapidly inactivated, (ii) the permeability to potassium increases near the crest of the action potential so that this ion can flow out and repolarize the fibre faster than at resting permeability (Hodgkin, Huxley & Katz, 1949). The prolonged depolarization that follows the rising phase of the action potential in cardiac muscle may therefore be attributed to (i) an incomplete and slow inactivation of the sodium permeability, (ii) an absence in the rise of potassium permeability (cf. Weidmann, 1951, 1956).

2. The Initiation of the Heart Beat

The electrical events leading to the initiation of the cardiac impulse have been described by Arvanitaki (1938). Exploring the snail's heart with surface electrodes, she found a region where a spontaneous depolarization developed during diastole. When this depolarization reached a critical value an impulse arose to travel through the heart. Bozler (1943) found a similar diastolic depolarization in the pace-maker region of the vertebrate heart, and it is now recognized that a slow loss in membrane potential between impulses is characteristic of most inherently rhythmic structures. A record obtained with an intracellular electrode from a spontaneously beating fibre in the sinus venosus of the frog's heart is reproduced in fig. 1. At no time is the membrane potential stationary: during diastole the potential falls gradually from a most negative value of 55 mv to about 40 mv. At this point the rate of depolarization increases greatly, and the inside of the fibre becomes positive by about 10 mv. The membrane is then recharged in the slow manner typical of cardiac muscle.

FIG. 1. ACTION POTENTIALS OF A PACE-MAKER FIBRE



Hutter & Trautwein (1956) by permission of Journal of General Physiology

Record obtained with an intracellular electrode from a frog sinus venosus beating spontaneously. The electrical events during four cardiac cycles are shown. Each cycle starts with a slow depolarization (upward deflection) during diastole. At a membrane potential of about 40 mv the rate of depolarization increases suddenly and the oscilloscope beam flies steeply upwards. The horizontal line indicates zero membrane potential.
Time scale: 5 dots represent 1 sec.

The depolarization in the diastolic interval has sometimes been called a pre-potential or pace-maker potential. In Purkinje's fibres, where an ectopic pace-maker usually develops after excision of the preparation, a relation between the concentration of sodium ions in the external medium and the rate of development of the pace-maker potential has been found (Draper & Weidmann, 1951; Weidmann, 1956). An inflow of sodium ions may therefore be responsible for the depolarization. The factors governing the net sodium flux during diastole are, however, not known.

3. Signs of Vagal Inhibition

a. Increase in Membrane Potential

Many years ago Gaskell (1887) showed that the demarcation potential of the arrested tortoise auricle may be increased by vagus and reduced by sympathetic nerve stimulation. This discovery at first received much attention, since it suggested that inhibition and excitation were attended by potential changes of opposite polarity. It soon became apparent, however, that a hyperpolarization of the auricle does not accompany vagal arrest under all experimental conditions. In the beating auricle of the tortoise, for instance, Gotch (1887) found no difference between the diastolic resting potential and the potential maintained during vagal arrest of the heart. Serious doubt about the significance of Gaskell's effect thus arose. The situation became still more confused by Einthoven & Rademaker's (1917) claim that the phenomenon was an artifact and, in spite of several later demonstrations of a hyperpolarization on vagal stimulation (Samojloff, 1914; Bayliss, 1915; Monnier & Dubuisson, 1934), Gaskell's important observations did not find a place in many text-books of physiology.

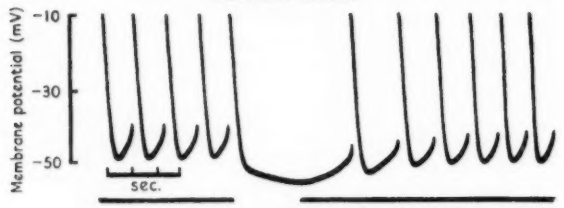
With the introduction of the intracellular electrode for the direct recording of membrane potentials, a new attack on the problem of vagal inhibition has been made. In a significant paper Burgen & Terroux (1953b) confirm Gaskell's original observation by showing that the resting potential of cat auricle fibres may be increased by acetylcholine and parasympathomimetic drugs. The hyperpolarization was greatest when the diastolic membrane potential was low compared with the equilibrium potential for potassium. Burgen and Terroux therefore suggest that the effect may be due, at least in part, to an increase in the permeability of the membrane to potassium ions. This hypothesis offers a satisfactory explanation for the inconstancy of the phenomenon: for only if the diastolic potential is less than the equilibrium potential for potassium, as may be the case when the permeability to other ions is high, can an increase in potassium permeability reveal itself in a rise of membrane potential.

b. Suppression of Pace-Maker Activity

Experiments on auricle fibres have thus given some insight into the mechanism of inhibition. But it is clear that the arrest of the heart by the vagus must be due to an action on the pace-maker fibres. Attempts have therefore been made to record the events during vagus stimulation in the sinus venosus of the frog and of the tortoise (Castillo & Katz, 1955a, 1957; Hutter & Trautwein, 1955, 1956) and in the sino-auricular node of the rabbit's heart (West, 1955; West, Falk & Cervoni, 1956). A similar picture was seen in all cases. Weak vagus stimulation or a small dose of acetylcholine slows the heart by reducing the rate of rise of the pace-maker potential; strong vagus stimulation suppresses the pace-maker potentials and arrests the heart by stabilizing the membrane potential at a value near the resting potential for auricle fibres. An increase in permeability towards such ions as potassium and chloride offers an explanation for these observations: for the more easily these ions can move across the membrane the less will be the depolarization caused by, say, an inward current of sodium during diastole.

The experiment illustrated in fig. 2 shows the suppression of pace-maker activity and the inhibitory polarization produced in a frog sinus venosus by a burst of vagal impulses. When single shocks are applied to the vagus a small increase in the membrane potential, reaching a peak in 1.5–2 sec. and lasting 5–7 sec., is seen after each shock (Castillo & Katz, 1955a, 1957). This slow time-course of the inhibitory potential is interesting because it has long been suspected that the concentration of acetylcholine acting on the pace-maker continues to increase for some time after each shock (Brown & Eccles, 1934). Whether it is due to a property of the post-ganglionic parasympathetic neurone, or to a diffusion delay after acetylcholine has been released, is, however, still unknown.

FIG. 2. INHIBITORY POLARIZATION DURING VAGAL STIMULATION



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The record is taken at an amplification that shows only the lower part of the action potential. During the fifth cycle, vagus stimulation at 20/sec. starts and continues for about 3 min. as indicated by the break in the reference line. This causes a suppression of pacemaker potentials and a gradual increase in the membrane potential (downwards deflection) to a value 9 mv higher than that usually reached during diastole.

c. Changes in the Action Potential: Block of Conduction

A well-known and invariably present sign of vagal activity is the shortening of the auricular action potential (Samojloff, 1914; Hoffman & Suckling, 1953; Burgen & Terroux, 1953b). This effect may also be explained by an increase of the membrane permeability to potassium: the greater the potassium permeability the sooner will the outflow of potassium from the depolarized fibre exceed the inflow of sodium, and the faster will the resting potential be restored to its diastolic value.

In the sinus venosus a few vagal volleys are sometimes enough to produce a striking shortening in the duration and a great reduction in the amplitude of the action potential (Hutter & Trautwein, 1956). The decrease in the amplitude of the action potential is accompanied by a slowing in the conduction velocity, and at the depth of vagal inhibition it appears impossible to set up a propagated response by direct shocks. Presumably the permeability to potassium and perhaps to chloride is then so high that little depolarization can be effected by the movement of other ions across the membrane. It seems possible that the block of conduction produced by the vagus at the auriculo-ventricular junction may be due to a similar mechanism. There the nerve-endings and receptors are localized in a strategic position through which the impulse must pass.

So far, the possibility that acetylcholine may reduce the action potential of sinus and auricle fibres by directly interfering with the excitatory process has not been considered. In theory such a mechanism could also account for the observed effects on the action potential, but it is not supported by experimental evidence. Thus an increase in the rate of rise of the action potential has been observed in auricle fibres treated with carbamylcholine (Burgen & Terroux, 1953b), and in the sinus the firing threshold is decreased during spontaneous recovery from vagus stimulation (Castillo & Katz, 1955a, 1957; Hutter & Trautwein, 1955, 1956). Both findings suggest that the excitatory mechanism is activated rather than inhibited by acetylcholine, presumably as a secondary consequence of the increase in membrane potential (Hodgkin & Huxley, 1952; Weidmann, 1955).[1]

d. Fall in Membrane Resistance

An increase in the ionic permeability of the resting membrane should cause a fall in the membrane resistance and hence in the time and space constants. The interpretation given to the changes in the pace-maker and action potentials may therefore be tested by studying the characteristic of the membrane with currents that are too weak to excite. This has been done by Trautwein, Kuffler & Edwards (1956) who found that the membrane resistance falls to about one-half when strips of frog auricle are treated with acetylcholine. A drastic reduction by acetylcholine in the electrical polarizability of the tortoise sinus venosus (Hutter, unpublished) and of the pace-maker fibres of the dog's heart (Trautwein, personal communication) has also been observed.

When the inhibitory action of acetylcholine on the heart is compared with its excitatory action on the neuromuscular junction, it becomes evident that the two actions are essentially of the same kind. In both cases a breakdown in the resistance of the post-junctional membrane occurs, and in both cases the form of the action potential is modified, although the process of electrical excitation is apparently not directly affected. The important difference is, of course, in the species of the ions whose movement is facilitated. It has been supposed that, at the neuromuscular junction, the increase in permeability is non-selective, for the potential at the end-plate tends to fall towards the low level that would obtain if sodium and most other ions were free to diffuse (Fatt & Katz, 1951; Castillo & Katz, 1955b). In the chemo-sensitive region of the heart, on the other hand, the increase in permeability must be restricted to potassium and chloride, for only these ions are distributed in a way that can lead to a firmer maintenance of the resting potential. How the permeability of the post-junctional membrane is altered by acetylcholine in either case is still unknown, but it has been suggested that the size of the permeability channel opened may be the factor determining whether excitation or inhibition occurs at a synapse (Coombs, Eccles & Fatt, 1955; Fatt, 1954).

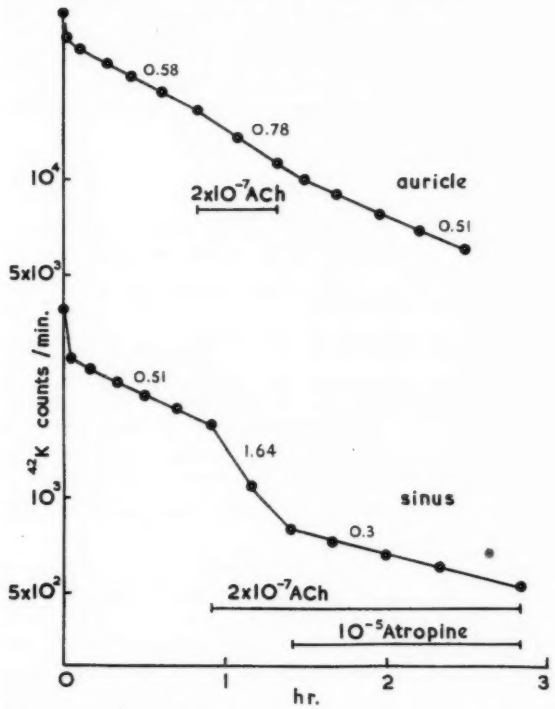
e. Increase in Potassium Permeability

The drastic changes in the action potential found in most parts of the sinus venosus during vagal stimulation, and the readily demonstrable breakdown in the membrane resistance, indicate that we are dealing with a very densely innervated tissue; a tissue where the receptors in the post-junctional membrane are so plentiful that their activation by the transmitter changes the properties of the membrane as a whole. It seemed likely, therefore, that the changes in permeability produced by acetylcholine in the sinus venosus might be large enough to be directly measured with the help of radioactive isotopes. This proved to be the case, and an appreciable increase produced by acetylcholine in both the inward and outward permeability to potassium has recently been reported (Harris & Hutter, 1956).

The result of an experiment in which the outflow of ^{42}K from a tortoise sinus venosus was studied is illustrated by the lower curve in fig. 3. It will be seen that, after an initial rapid phase of loss, the outflow of ^{42}K proceeded at an apparently constant rate. After about an hour the preparation was treated with acetylcholine. This stopped the beats and accelerated the outflow of ^{42}K more than three times. When the inhibition was removed by atropine the outflow of ^{42}K again slowed. For comparison, a similar experiment on the right auricle of the same heart is shown in the upper curve. Here also ^{42}K was lost more quickly under the influence of acetylcholine, but it is clear that the response was not nearly as great as in the sinus venosus. Presumably fewer receptors

[1] See also Burn, p. 181 of this number of the Bulletin.—Ed.

FIG. 3. ACTION OF ACETYLCHOLINE ON THE OUTFLOW OF POTASSIUM (unpublished illustration of an experiment by Harris & Hutter, 1956)



An experiment on the isolated sinus venosus (lower graph) and right auricle (upper graph) of a tortoise heart. The tissues were loaded with ^{42}K and the rate of loss of the isotope was studied.

The small figures give the rate constants in hr.$^{-1}$ at the different stages of the experiment. In the sinus venosus the action of acetylcholine was stopped by atropine.

Abscissae: time (hr.)

Ordinates: isotope content of tissue in counts/min. plotted on a logarithmic scale

were present, so that the effect of acetylcholine was swamped by the large ionic interchange that goes on all the time across the rest of the membrane. In some experiments the rate of ^{42}K loss was tested in potassium-rich solutions, i.e., the concentration of potassium in the inactive wash solution was raised from 2.6 to between 9 and 90 mM/l. Under these circumstances acetylcholine increased the rate of loss of ^{42}K as much as six or seven times. This may be the true extent of the increase in permeability, and the somewhat smaller effect under normal conditions may be due to the restraint imposed on the outflow of potassium by the increase in membrane potential produced by acetylcholine.

Whether the permeability to chloride is also increased by acetylcholine is an important question that has not yet received an answer.

4. Events during Sympathetic Acceleration

Recent experiments (Hutter & Trautwein, 1955, 1956; West *et al.* 1956) have confirmed that the acceleration of the heart by sympathetic stimulation is due to a faster depolarization of the membrane during diastole. The threshold for impulse propagation is therefore reached sooner during each cycle, and beat follows beat in closer succession (fig. 4). As the frequency increases, the amplitude of the action potential grows to a greater height. The electrical response of the pace-maker fibres to sympathetic stimulation thus stands in obvious and not surprising contrast to that produced by vagus stimulation.

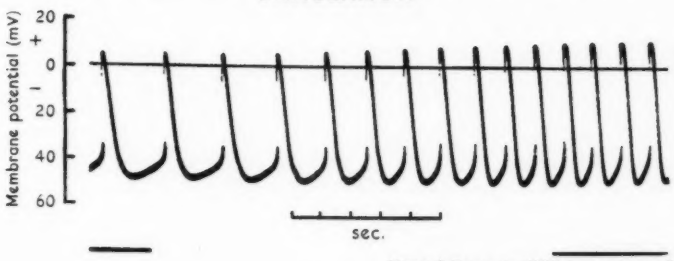
The effect of sympathetic stimulation has also been studied in arrested preparations. Here a species difference has become apparent. In the sinus venosus of the frog, arrested by previous stimulation of the intracranial vagus, sympathetic stimulation causes a fall in the membrane potential (Castillo & Katz, 1955a, 1957). This effect recalls the depolarization seen under similar circumstances in the tortoise auricle by Gaskell. In isolated strips of dog auricle, on the other hand, treatment with adrenaline caused a consistent and sometimes striking increase in the membrane potential (Dudel & Trautwein, 1956). At the moment it is therefore difficult to discuss the action of adrenaline in general terms.

Considering then only the acceleration and depolarization seen in the cold-blooded heart, one might postulate that adrenaline acts exactly in the reverse way to acetylcholine, viz., that it decreases the permeability to potassium. No evidence in favour of this hypothesis has, however, been obtained in experiments with ^{42}K (Harris & Hutter, unpublished). There remains then the possibility that adrenaline has a facilitating effect directly on the excitatory mechanism. If, for example, more sodium carriers were made available through its action, the increase in the slope of the pace-maker potential and in the amplitude of the action potential could both be explained.

5. Summary

The pace-maker fibres of the heart are characterized by an unstable membrane potential. During diastole a spontaneous depolarization develops which on reaching a critical value sets up an impulse that travels through the heart. The arrest

FIG. 4. ACTION POTENTIALS DURING SYMPATHETIC STIMULATION



Hutter & Trautwein (1956) by permission of Journal of General Physiology

Frog sinus venosus treated with atropine 1 in 10^6. Stimulation of vago-sympathetic trunk as indicated by break in reference line. Note the increase in the slope of the pace-maker potentials and in the amplitude of the action potentials.

of the heart by the vagus is due to a suppression of this spontaneous activity, and to a stabilization of the membrane potential at a sub-threshold value. It seems likely that vagal inhibition is due to an action of acetylcholine on the post-junctional membrane, whereby the membrane permeability to the ions maintaining the resting potential, i.e. potassium and perhaps also chloride, is increased. Thus a fall in the membrane resistance has been observed on treatment of pacemaker and auricle fibres with acetylcholine, and direct evidence for an increase in the inward and outward permeability to potassium has been obtained with the help of the radioactive isotope ^{42}K. The well-known decrease in the duration of the auricular action potential and the block of conduction at the auriculo-ventricular junction may also be due to an enhanced permeability of the resting membrane to potassium ions. Sympathetic stimulation accelerates the heart by increasing the rate of development of the slow depolarization during diastole.

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ACETYLCHOLINE AND CARDIAC FIBRILLATION

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1 The action of acetylcholine on the atria
2 Choline acetylase
3 Production of fibrillation
4 Mechanism of action of acetylcholine
5 The action of adrenaline
6 Fibrillation in isolated atria
7 Influence of potassium concentration
8 Loss of potassium in fibrillation
9 Action of quinidine
10 The conflicting actions of acetylcholine
References

Work on the autonomic nervous system has concentrated for many years on the problems of the transmission of impulses at nerve junctions and nerve-endings. Recently, however, attention has been drawn to the part played in the tissues by the same substances which act as chemical transmitters, a part which is independent of the extrinsic nerves. Evidence has been published concerning the formation and liberation of acetylcholine in rabbit intestine (Feldberg & Lin, 1949, 1950) and in the gill plates of *Mytilus edulis*, where it controls the ciliary movement (Bülbring, Burn & Shelley, 1953).

This paper begins with a discussion of similar evidence obtained in cardiac tissue, and then passes on to describe work on cardiac fibrillation which has arisen out of it.

1. The Action of Acetylcholine on the Atria

The atria of the rabbit heart can be dissected free from other tissue, and, when suspended in an isolated-organ bath, will beat spontaneously for many hours. Some years ago it was observed that in various circumstances the contractions were arrested, but would then begin again if acetylcholine was added to the bath (Burn & Vane, 1949; Bülbring & Burn, 1949; Briscoe & Burn, 1954). Since acetylcholine normally inhibits the contractions, and increases the polarization of the atrial cell (Gaskell, 1887; Burgen & Terroux, 1953; Hutter & Trautwein, 1956), it was difficult to understand how it could exert what appeared to be a depolarizing action.

The clue to this mystery was provided by the work of Marshall & Vaughan Williams (1956) who found that, when isolated atria were cooled to a temperature below 20° C., their contractions ceased, and that the addition of acetylcholine in a concentration of 10^{-7} g./ml. caused them to start again. They recorded the action potential by external electrodes from a point near the pace-maker, and also from the tip of the left auricle. They found that, in the great majority of their preparations, when the mechanical activity failed as the temperature fell below 20° C., the action potential disappeared in the left auricle; at the pace-maker, however, small potentials could still be recorded. When acetylcholine was added, then, after a delay of 1–2 min., normal action potentials were seen to "take off" from the small pace-maker potentials, and were propagated in the usual way. Unless the pace-maker potentials were present, acetylcholine was without effect.

From these results it was clear that acetylcholine was not causing contraction by depolarization. Marshall (1957) has now observed that at the low temperature the resting potential within the atrial cell fell to a point at which the impulses from the pace-maker were no longer capable of causing depolarization. Acetylcholine raised the resting potential, and thus made the pace-maker impulses effective.

In 1949 Bülbring & Burn found that, when atria were left to beat in the isolated-organ bath for 30 hr. or more, the contractions became slower and finally ceased. The addition of acetylcholine in concentrations from 10^{-8} g./ml. upwards started the contractions again. Recently it has been found that there was a progressive fall in the amount of potassium in the atria when they were left in the bath for so long a period. This indicated that the contractions stopped because this loss of potassium eventually reduced the resting potential below a threshold value, and rendered the pace-maker impulses ineffective (Burn & Goodford, unpublished). In this case, too, acetylcholine would restart the contractions by raising the resting potential above the threshold value.

The effect of cooling has also been studied in a preparation of atria with the vagus nerves attached, as described by McEwen (1956). When the temperature was not lower than 22° C., the contractions continued and vagal stimulation caused inhibition. At a temperature between 22° C. and 15° C. atrial contractions almost ceased, and vagal stimulation then excited contractions. See fig. 1 (Burn & Rand, 1957). There is no reason to doubt that this observation is explained in the same way as the action of acetylcholine already discussed. At the low temperature the resting potential had fallen to a point at which the pace-maker impulses were ineffective in causing depolarization. When the vagus nerves were stimulated, acetylcholine was released and it raised the resting potential above the threshold. The excitatory effect was abolished by atropine.

In summary it can be said that the excitatory actions of acetylcholine which have been considered are not depolarizing actions, and that on the contrary they are exerted by virtue of the power of acetylcholine to increase the permeability of the atrial cell membrane to potassium ions and so to raise the resting potential towards the equilibrium potential for potassium.

2. Choline Acetylase

In 1946 Comline showed that the choline acetylase system was present in rabbit atria. This enzyme system forms acetylcholine, and its activity in atria can be measured by making a powder from the atria with ice-cold acetone; the powder is then incubated with choline, sodium citrate and adenosinetriphosphate according to the procedure of Feldberg & Mann (1946). The acetylcholine formed is estimated on the frog rectus muscle.

Evidence of the liberation of acetylcholine in hearts free from vagal influences has been obtained in various experiments (Briscoe & Burn, 1954; Burn & Walker, 1954; Burn,

Vaughan Williams & Walker, 1956; Day, 1956). Thus when the isolated rabbit heart was perfused by the Langendorff method with Locke's solution containing eserine, and the perfusate coming from the heart was recirculated through it, a substance indistinguishable from acetylcholine accumulated in the perfusate. The perfusate itself, diluted to the required tonicity, caused a contraction of the leech muscle which was progressively greater as the recirculation continued. When the perfusate was freeze-dried, an extract of the residue caused a fall of blood pressure in the cat, inhibition of the frog heart and contraction of the frog rectus muscle. Evidence is thus available not only that acetylcholine can be formed in atria but also that it is formed.

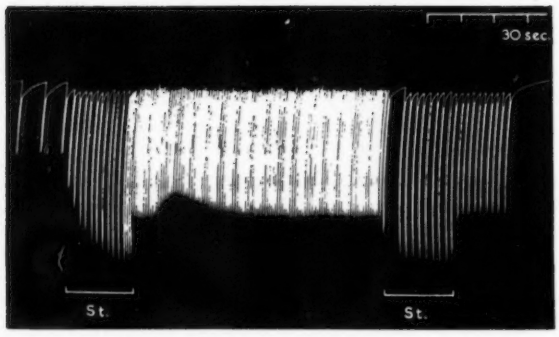
In view of the action of acetylcholine on the resting potential, it is very likely that this constantly formed acetylcholine affects the resting potential of the atria, and it is possible that one of its functions is to maintain the resting potential at a suitable level.

The choline acetylase activity was studied in atria freshly excised from the body, and was compared with that of atria which had ceased to beat after being left in the bath for upwards of 30 hr., and also with that of atria which, having ceased to beat, had been restarted by adding acetylcholine to the bath. The respective mean results were (i) 46 μg./g. powder/hr., (ii) 7 μg./g. powder/hr. and (iii) 37 μg./g. powder/hr. The figures demonstrated that there was some connexion between atrial contractions and choline acetylase activity, and that atria which were not beating had lost much of their power to form acetylcholine; they regained it, however, when the contractions were started again. Another result of interest for this discussion was as follows. When an acetone-dried powder was prepared from atria which had ceased to beat, the synthesis of acetylcholine by this powder was increased when acetylcholine was added, the increase being in proportion to the amount added (Bülbring & Burn, 1949).

In the study of vagal stimulation of atria at low temperature, in some preparations contractions were observed only during application of the stimulus; in others, however, (see fig. 1) the contractions persisted and a normal rhythm was restored. The restoration of this normal rhythm may have been due to the effect of the acetylcholine in reactivating the choline acetylase mechanism as observed in vitro. In support of this was the experience with atria which had ceased to beat after many hours in the bath. When restarted by acetylcholine, they stopped again if the acetylcholine was quickly washed out. If, however, the acetylcholine was left in the bath for some time, the beat was not arrested when the acetylcholine was washed out. Thus acetylcholine might restore the resting potential (i) directly and also (ii) by reactivating the choline acetylase system.

A final result with acetone-dried powders prepared from atria was that, when freshly excised atria were used, the addition of acetylcholine diminished the synthesis of acetylcholine in proportion to the amount added. This observation may be related to the effect of vagal stimulation seen in fig. 1 when a normal rhythm had been established. Instead of a transient inhibitory action, not outlasting the period of stimulation, the inhibition was followed by arrest of the beat. If we suppose, in accordance with the in-vitro observations, that during the normal rhythm the choline acetylase system was active, the activity was not likely to have been much above threshold, since the temperature was only 16.5° C. The inhibitory effect of the acetylcholine from the vagus may then have diminished this activity to sub-threshold level, and so arrested the beat.

FIG. 1. MECHANICAL RECORD OF CONTRACTIONS OF ISOLATED ATRIA



At St., vagal stimulation for 1 min. increased the rate and amplitude and restored a regular beat; 4 min. later a second period of vagal stimulation caused inhibition and led to cessation of the beat.

Temperature: 16.5° C.

3. Production of Fibrillation

In the course of experiments on the heart-lung preparation it was found that, when the atria were stimulated electrically during the infusion of acetylcholine, the atria went into fibrillation (Burn, Vaughan Williams & Walker, 1955). The rate of infusion of acetylcholine which produced this effect varied in different preparations from 40 μg./min. to 4 mg./min., the mean rate being 1 mg./min. The rate of stimulation necessary to produce fibrillation was as low as 200/min. in some preparations, but in others was as high as 750/min. If the stimulation was applied for 30 sec., fibrillation was produced, and it then persisted so long as the infusion of acetylcholine was maintained. The longest period tested was 1.5 hr. Normal rhythm reappeared usually within 1 min. of stopping the infusion. Fibrillation could be produced, maintained and then stopped at will, many times in an experiment.

4. Mechanism of Action of Acetylcholine

An approach to the mechanism by which acetylcholine maintains fibrillation can be made by considering its effect on the action potential. Using intracellular electrodes, Burgen & Terroux (1953) and Hoffman & Suckling (1953) showed that the normal action potential of the atrium consisted of an abrupt fall from 90 mv overshooting to 30 mv, followed by a slow repolarization complete in about 150 msec. Under the influence of acetylcholine, however, the repolarization was accelerated, and might be completed in only 10 msec. in the presence of a high concentration. Acetylcholine thus greatly shortens the period in which the fibres are inexcitable, and it becomes at once evident that, if a brief application of electrical stimulation at a high rate leaves fibres slightly out of phase, then, in the presence of acetylcholine, fibres may stimulate one another continuously. If the acetylcholine is withdrawn, the rate of repolarization will be slowed and continuous re-excitation will become impossible. In support of this approach to fibrillation in terms of the action potential, is Hoffman and Suckling's observation that acetylcholine did not affect the

ventricular action potential. In studying ventricular fibrillation in the isolated rabbit heart, Armitage, Burn & Gunning (1957) found that neither carbachol nor eserine facilitated it, nor did atropine arrest it.

5. The Action of Adrenaline

Webb & Hollander (1956) showed that, in the presence of adrenaline, the atrial action potential in the rat was altered so that repolarization occurred more slowly. This suggested that adrenaline might antagonize the action of acetylcholine in producing atrial fibrillation. Adrenaline is generally understood to favour ventricular fibrillation, and this has been observed in the isolated rabbit heart (Burn & Goodford, unpublished). In experiments in which acetylcholine was infused into the heart-lung preparation alternately alone and then together with noradrenaline or adrenaline, it was found that stimulation that precipitated atrial fibrillation when acetylcholine was infused alone failed to do so when acetylcholine and noradrenaline were infused together (Burn, Gunning & Walker, 1957).

6. Fibrillation in Isolated Atria

Fibrillation was also produced in the isolated atria of the rabbit by electrical stimulation in the presence of acetylcholine, and the fibrillation persisted for periods of 10–45 minutes after stimulation (Holland & Burn, 1957). This fibrillation occurred only when the potassium concentration of the solution in which the atria were suspended was reduced to 100 mg./l. (approx. 1.4 mM/l.).

Hollander & Webb (1955) have shown that a reduction of temperature prolongs the duration of the action potential in the rat, and the effect of temperature on fibrillation in isolated atria was therefore studied. It was found that, when stimulation in the presence of acetylcholine produced fibrillation at 37° C., it failed to do so at 29° C. (Beaulnes & Day, 1957).

7. Influence of Potassium Concentration

The importance of potassium concentration for the occurrence of fibrillation became evident both for atrial and for ventricular fibrillation. When Burgen & Terroux (1953) described the shortening of the atrial action potential by acetylcholine, they suggested that it might act by making the cell membranes more permeable to potassium. The effect of a slow infusion of KCl on atrial fibrillation in the heart-lung preparation was therefore determined. Fibrillation during the infusion of acetylcholine was arrested when a mean amount of 225 mg. KCl had been added to 1 litre of blood, and thereafter stimulation failed to produce fibrillation except while it was applied (Burn, Gunning & Walker, 1956). This observation was consistent with the view that the shortening of the action potential by acetylcholine was accompanied by an increased loss of K^+ through a more permeable cell membrane, and further that such an increased loss was an important factor in fibrillation. For by a small rise of the external concentration of K^+ up to 1.6 times normal, whereby the replacement of K^+ within the cell was facilitated, fibrillation was arrested. This line of argument was greatly strengthened by the direct demonstration by Harris & Hutter (1956) that acetylcholine increased the permeability to K^+ of the sinus and atria of the frog and the tortoise.[1]

[1] See also Hutter, p. 176 of this number of the Bulletin.—ED.

8. Loss of Potassium in Fibrillation

The importance of potassium loss was emphasized by the conditions required for fibrillation in the isolated atria. Even in the presence of acetylcholine, stimulation did not cause fibrillation unless the external K^+ concentration was reduced to 1.4 mM/l. Holland, Burn & Schümann (1957) studied the effect of stimulation on loss of K^+. They applied stimulation at rates from 200/min. to 1,200/min. In the absence of acetylcholine, the rate of K^+ loss did not change greatly as the rate of stimulation rose. At 200/min. the rate of K^+ loss was 3.8 mM/kg. wet weight/5 min., and at 1,200/min. it had risen to 4.7 mM. When acetylcholine was added (3×10^{-4} g./ml.), the K^+ loss at 200/min. was 6.8 mM, and as the rate of stimulation rose to 800/min. the K^+ loss rose to 10.5 mM. When the K^+ loss reached this value, fibrillation was observed. These results suggested that fibrillation occurred when the K^+ loss per unit of time reached a certain value.

9. Action of Quinidine

Quinidine is the substance which has long been used to arrest atrial fibrillation. Dawes (1946) pointed out that it was an antagonist of acetylcholine in skeletal muscle, in smooth muscle and in cardiac muscle. Its action in fibrillation is commonly stated to be due to the prolongation of the refractory period.

When quinidine was allowed to act on the isolated atria of the rabbit, it decreased the amplitude and rate of contractions until they stopped. If acetylcholine was added to the bath the contractions began again (Briscoe & Burn, 1954). In view of the foregoing evidence that acetylcholine makes the membrane more permeable to K^+, the observation suggested that quinidine might arrest the contractions because it had the opposite effect, diminishing the permeability of the cell membrane to K^+, until the flux of ions across the membrane ceased.

Recently Armitage (1957) showed that, when the contractions of the atria were diminished in rate and in size by quinidine, they increased again on reducing the concentration of K^+ in the bath to one-half or one-quarter. Even if the contractions had been arrested, such a reduction led to the resumption of the beat. This evidence supported the view that quinidine reduced the membrane permeability for potassium and that the flux required for normal contraction could be restored by lowering the external K^+ concentration.

In order to provide direct evidence for this conclusion, in a non-contractile structure, studies were made on the uptake of K^+ by red cells. Human blood was taken and stored for 3–5 days at 0° C. During the storage, the metabolic processes were arrested, and the concentration of K^+ within the cells (measured in relation to the haemoglobin) was observed to fall. At the end of this time the blood was incubated for several hours at 37° C., and the cells then gained potassium from the plasma. The rise of K^+ within the cells was determined in control blood, and in blood to which quinidine was added. The observation was made in all experiments that the uptake of K^+ was depressed by quinidine (Kärki, Burn & Burn, 1957).

10. The Conflicting Actions of Acetylcholine

The characteristic feature of cardiac muscle is the long duration of the action potential which lasts for 150 msec. or more, as compared with a few milliseconds for skeletal

muscle. There can be little doubt that this long duration is a device for prolonging the period of inexcitability after a contraction, in order that fibres which are out of phase shall not stimulate one another.

Acetylcholine breaks down this protective device in the atria by facilitating the efflux of K^+, so that, when stimuli arrive at a high rate, fibrillation occurs. It is therefore of no small importance that the substance which has this effect should have an inhibitory action on the pace-maker and on atrioventricular conduction. For, while acetylcholine makes the membrane more permeable and unstable, it prevents impulses from reaching it except at a slow rate. Similarly, by its action on the atrioventricular bundle, it protects the ventricles from a breakdown in the atria.

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PHARMACOLOGICAL PROPERTIES OF SENSORY RECEPTORS AND THEIR RELATION TO THOSE OF THE AUTONOMIC NERVOUS SYSTEM

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1 Excitation of sensory receptors by acetylcholine and related substances
2 Effects of blocking agents and anticholinesterases
3 Sensory receptors and the autonomic nervous system
References

Peripheral synapses in the autonomic nervous system behave in a characteristic manner when they are acted on by acetylcholine, nicotine or drugs which modify the effects of these substances. Reactions to these substances in whole organs have often been interpreted as indicating the presence of such synapses. The purpose of this review is to show that many sensory receptors resemble autonomic synapses in their response to these drugs and that this fact must be taken into account whenever the action of such drugs on a tissue is being assessed. There is, however, one very important difference between synapses and receptors in respect of the action of acetylcholine; at cholinergic synapses acetylcholine is the physiological transmitter, but at receptors acetylcholine appears to have no physiological function as an intermediary in the initiation of nerve impulses. While the greater part of this review will be devoted to those pharmacological properties of receptors that are related to the action of acetylcholine, some mention will also be made of the effects of adrenaline on sensory receptors.

1. Excitation of Sensory Receptors by Acetylcholine and Related Substances

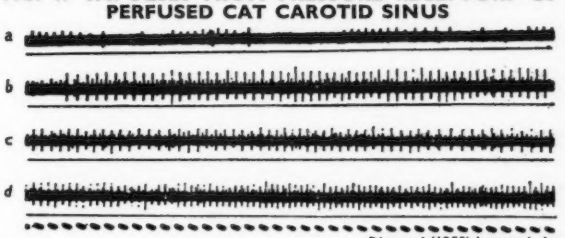
Mechano-Receptors

A variety of receptors, whose physiological stimulus is mechanical in nature (e.g., stretch or pressure receptors), have been shown to be sensitive to acetylcholine or nicotine. The effect was first demonstrated in the skin and mesentery of cats and dogs (Brown & Gray, 1948). Nerve impulses were recorded from a suitable afferent nerve while the drugs were applied by the technique of close arterial injection; in this technique the blood supply is cut off for a short time, and the vascular bed of the piece of tissue in question is momentarily filled with the solution of the drug. Injections of 0.2 ml. of a solution of acetylcholine in a concentration of 10^{-5} g./ml. caused a discharge of nerve impulses that lasted for 10 sec. In suitable records it could be seen that nerve fibres that had been previously silent were excited by the drug (Douglas & Gray, 1953). While it is possible that sensory units sensitive to several different forms of energy were involved in these discharges, it is certain from an analysis of the recorded size of the impulses that receptors sensitive to mechanical energy were excited.

A more detailed analysis of the excitation of mechano-receptors by acetylcholine has been made by recording from single sensory units in the perfused carotid sinus (Diamond, 1955) and from single fibres from frog's skin (Jarrett, 1956). The experiments on the carotid sinus are illustrated in fig. 1. The first and third records show the response of the unit to two pressures, one above and the other below threshold. The second record was obtained at the same pressure as the first record, but in this instance the perfusion fluid contained acetylcholine. In such an experiment there can be no doubt that the unit is one sensitive to mechanical stimuli and that a previously silent unit has been caused to fire. This technique of recording from single units from the perfused carotid sinus made it possible to relate the frequency of impulses in the afferent nerve fibre to the concentration of acetylcholine in the perfusion fluid. Such an experiment showed that, over the range tested, the frequency of impulses was related to the logarithm of the concentration of the drug. Experiments with single units have also shown that summation can occur between a mechanical stimulus and the action of the drug; thus, in the carotid sinus preparation, the frequency of impulses from a pressure-sensitive unit depends on the pressure in the sinus and the concentration of acetylcholine in the perfusion fluid (fig. 1). The property of summation has been shown particularly well in the experiments with single fibres from frog's skin (Jarrett, 1956). In these experiments the thresholds of the rapidly adapting mechano-receptors of the skin were measured by means of short mechanical pulses from a crystal transducer; it was found that acetylcholine in the bath consistently lowered the threshold to mechanical stimulation. A larger dose of acetylcholine excited impulses in the absence of any mechanical stimulus.

So far it has been assumed that the action of acetylcholine

FIG. 1. IMPULSES FROM PRESSURE RECEPTORS OF PERFUSED CAT CAROTID SINUS



Diamond (1955) by permission of Journal of Physiology

Records begin 9.5 sec. after each injection.
a: sinus pressure, 25 mm. Hg; injection, 0.5 ml. saline
b: sinus pressure, 25 mm. Hg; injection, 0.5 ml. acetylcholine 10^{-4} g./ml.
c: sinus pressure, 111 mm. Hg; injection, 1.0 ml. saline
d: sinus pressure, 111 mm. Hg; injection, 1.0 ml. acetylcholine 10^{-5} g./ml.
Threshold sinus pressure 88 mm. Hg
Time: 0.1 sec.

has been on the receptor or at least on the sensory pathway, and that the effects described were not secondary to stimulation of any part of the autonomic nervous system. There is considerable evidence that this assumption is justified. The direct action of acetylcholine on smooth muscle is abolished by atropine and is not imitated by nicotine or by carbaminoylcholine. In contrast, the acetylcholine action on sensory receptors is unaffected by atropine and is imitated by nicotine, lobeline and carbaminoylcholine. Furthermore, when a substance, such as adrenaline, which stimulates smooth muscle of vessels and hair follicles is used to activate mechano-receptors, the longer latency and slower time-course of the response clearly distinguish it from the rapid effect of acetylcholine and nicotine. This last observation also argues against the possibility that the drugs are exciting sympathetic synapses and thus causing contraction of smooth muscle; this possibility has been excluded by completely blocking the effects of sympathetic nerve stimulation or of injected adrenaline with an adrenaline-blocking agent and finding that the acetylcholine response has been unaffected by such treatment (Douglas & Gray, 1953; Diamond, 1955).

Besides those mechano-receptors already considered, there are others that have been shown to be sensitive to acetylcholine or to nicotine; they are the stretch receptor of the crayfish (Wiersma, Furshpan & Florey, 1953), mechano-receptors of the cat's tongue (Landgren, Liljestrand & Zotterman, 1954), and those of the stomach (Paintal, 1954) and lung (Paintal, 1955). A closely related substance, succinylcholine, has been shown to act on the stretch receptors of the cat's muscle spindle (Granit, Skoglund & Thesleff, 1953).

Other Types of Receptor

The chemo-receptors of the carotid body of the cat were the first sensory structures to be shown to be excitable by acetylcholine or nicotine, and this organ is particularly sensitive to these substances (Samaan & Stella, 1935; von Euler, Liljestrand & Zotterman, 1941). This high sensitivity is probably due to the particularly close relationship that exists between the blood and the cells of the organ. Unlike the other receptors considered in this review, the receptors of the carotid body consist of two parts: the glomus cell, which probably has a sensory function, and the nerve terminals themselves, which lie in close relation to the glomus cells. For this reason there has been much discussion about the site at which the drugs act and about the possibility that acetylcholine is a synaptic transmitter.

Receptors responding to temperature have been shown to be sensitive to acetylcholine by recording impulses in afferent nerve fibres from the cat's tongue while applying the drug to the surface of the organ (Dodt, Skouby & Zotterman, 1953). The sensation of cold has been modified by the intracutaneous injection of acetylcholine (Bing & Skouby, 1950). The threshold for the sensation of pain has been lowered by intracutaneous injections of acetylcholine (Skouby, 1951). More dramatic, however, is the production of pain by the application of acetylcholine to the exposed base of a cantharidin blister (Armstrong, Dry, Keele & Markham, 1953). This is a phenomenon that is remarkably consistent: a given dose producing a characteristic transient pain, which, on each occasion in any one subject, reaches the same maximum intensity as recorded on a subjective scale.

Indirect evidence of the effect of acetylcholine on receptors has come from a different type of experiment (Coon & Rothman, 1940, 1941); in fact these experiments were some of the earliest to be done in this field. Intradermal injections of acetylcholine were found to set up pilomotor and sweating responses around the site of injection, though too far from the injection bleb to be due to a direct action of the drug. The effect was abolished by degeneration of the sympathetic supply to the region, but not by acute section of these nerves. These authors concluded that the acetylcholine excited peripheral receptors and that the response was the result of an axon reflex.

There is thus considerable evidence that sensory receptors of many different types are sensitive to acetylcholine and related compounds. The word receptor has been used throughout, though we cannot be absolutely certain that the drug acts on that part of the axon membrane that is involved in the transducer mechanism. It is probable that this is so, as comparable applications of the drugs to nerves, and even to non-myelinated terminals such as the preganglionic terminals in sympathetic ganglia, are without any detectable effects (Brink, Bronk & Larrabee, 1946; Brown & MacIntosh, 1939; Lorente de Nó, 1944; Hodgkin, 1947; Jarrett, 1956). Further discussion on the association of the drug sensitivity with other special features of receptors is perhaps out of place in a symposium on the autonomic nervous system.

2. Effects of Blocking Agents and Anticholinesterases

Acetylcholine and Nicotine

Large doses of acetylcholine or nicotine abolish the response to a subsequent injection of an exciting dose of the drug. In the cat's skin and carotid sinus preparations, this blocking action does not alter the response of the receptors to mechanical stimulation. In the frog's skin (Jarrett, 1956) and cat's tongue preparations (Landgren, Liljestrand & Zotterman, 1954), on the other hand, the response to mechanical stimulation is also blocked. It seems likely on present evidence that the action of acetylcholine is to depolarize the axon terminal and that, if this depolarization is sufficiently great and lasts sufficiently long, it is bound to stop all electrical activity in the terminal. It is surprising, then, that the responses to mechanical stimulation of the cat's skin and carotid sinus preparations are not affected at a time when the response to a subsequent dose of acetylcholine is blocked. The explanation of this phenomenon probably lies in the dissociation of the chemical blocking action and depolarizing effects of nicotine and acetylcholine that has been shown to occur for nicotine at the synapse of the sympathetic ganglion (Paton & Perry, 1953) and for acetylcholine at the neuromuscular junction (Thesleff, 1955).

Curarine and Hexamethonium

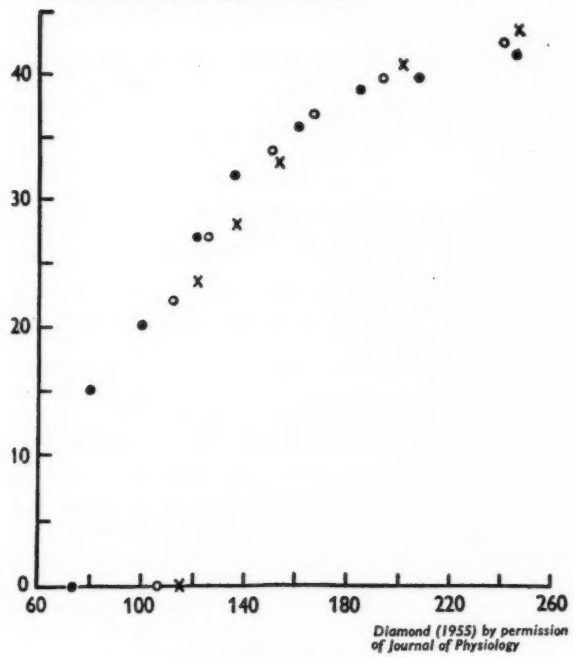
These blocking agents are not, in other situations, known to cause any change in membrane potential. For this reason their action on this response of sensory receptors to acetylcholine-like substances is of particular interest. One or other of these agents has been shown to block this response of mechano-receptors in the cat's skin (Brown & Gray, 1948; Douglas & Gray, 1953), carotid sinus (Diamond, 1955), and in frog's skin (Jarrett, 1956), and the response of chemo-receptors in the cat's carotid body (Douglas, 1952). In all instances doses of the blocking agent that prevent the response to a subsequent injection of acetylcholine have no effect on the response of the receptor to its natural stimulus. Relatively

large doses of hexamethonium have been shown to cause some diminution in the discharge of impulses in the sinus nerve in response to oxygen lack (Landgren, Liljestrand & Zotterman, 1953). On the other hand very large doses of hexamethonium do not affect the responses of the carotid sinus receptors to pressure (Diamond, 1955). The pressure–frequency relations of single units from the cat's carotid sinus have been measured before and during perfusion with concentrations of hexamethonium as great as 1%; even though perfusion with these high concentrations was continued for some hours, no significant change in the pressure–frequency relation was observed (fig. 2). This concentration of hexamethonium was about ten thousand times that required to block the action of acetylcholine.

Eserine

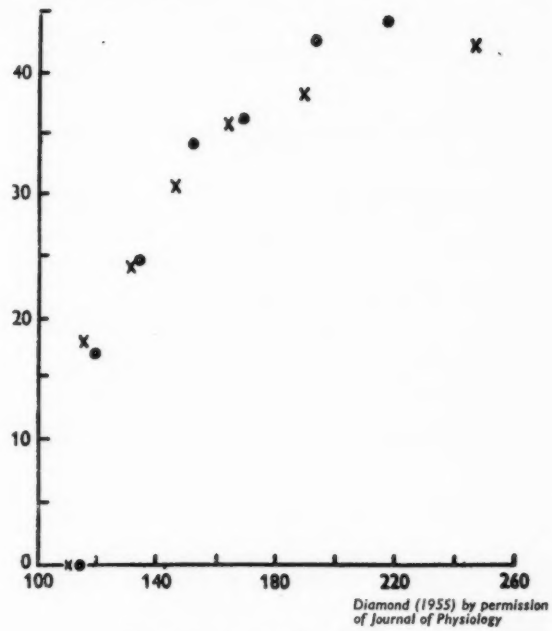
This anticholinesterase has been shown to potentiate the effects of subsequent applications of acetylcholine in several of the preparations described. However, close arterial injections of eserine into cat's or dog's skin had no effect on the activity in the sensory nerve (Brown & Gray, 1948). A similar result has been obtained with the perfused cat's carotid sinus by adding 10^{-5} g./ml. eserine to the perfusion fluid and measuring the pressure–frequency relation of a single unit before and during the time eserine was present; no change in the response of the unit was found (fig. 3). The local application of relatively large doses of eserine or other cholinesterase-

FIG. 2. RELATION BETWEEN PRESSURE IN PERFUSED CAT CAROTID SINUS AND IMPULSE FREQUENCY OF A SINGLE SENSORY UNIT



Abscissae: pressure (mm. Hg)
Ordinates: impulses/sec.
o and x: controls
●: 1% hexamethonium in perfusion fluid

FIG. 3. RELATION BETWEEN PRESSURE IN PERFUSED CAT CAROTID SINUS AND IMPULSE FREQUENCY OF A SINGLE SENSORY UNIT



Abscissae: pressure (mm. Hg)
Ordinates: impulses/sec.
●: control
x: eserine 10^{-5} g./ml. in perfusion fluid

inhibiting substances to the carotid body, however, was found to enhance the activity of the chemo-receptors (Landgren, Liljestrand & Zotterman, 1952), though the exact significance of these results and those involving blocking agents has not been elucidated (Daly, 1954). Similar effects have been reported on chemo-receptors in the cat's tongue (Landgren *et al.* 1954).

Adrenaline

Adrenaline is another substance, relevant to the autonomic nervous system, that has been shown to affect the activity of sensory receptors, though the work that has been done on this substance is far less extensive than that done on the acetylcholine-like group of drugs. The sensitivity of mechano-receptors in the frog's skin to stimulation by mechanical pulses has been shown to increase if the preparation is bathed in a solution containing 10^{-6} g./ml. adrenaline or if the sympathetic supply to the skin is stimulated (Loewenstein, 1956). The most convincing evidence that this effect is a direct one on the receptor comes from experiments in which the receptor potential of the Pacinian corpuscle has been recorded while the end-organ has been soaked in a solution containing adrenaline (Loewenstein & Altamirano-Orrego, 1956); in this instance the effect of adrenaline was to increase the amplitude of the receptor potential resulting from a given stimulus and to increase the response to a single pulse from the usual single impulse to many impulses.

Adrenaline has also been shown to excite or increase the afferent discharge from cat's skin (Douglas & Gray, 1953) and from cat's carotid sinus (Landgren, Neil & Zotterman, 1952; Diamond, 1955); these effects, however, are almost certainly due to the action of adrenaline on the pilomotor muscles of the skin and on the smooth muscle in the wall of the sinus.

3. Sensory Receptors and the Autonomic Nervous System

As classically defined, the autonomic nervous system is an efferent system, but the activity of this system is determined by afferent activity. Much reflex activity in the autonomic nervous system involves the central nervous system, but some reflex arcs are entirely peripheral (Brown & Pascoe, 1952); there are also responses of a type usually associated with the autonomic nervous system, which are brought about through axon reflexes. It is clear, therefore, that investigations into the activity of the autonomic nervous system may necessitate a consideration of the activity of the afferent side. A knowledge of conditions likely to influence the activity of receptors and thus to initiate or modify autonomic effects is clearly of importance. Since the forms of energy that excite receptors under physiological conditions are well known, this article has been devoted to a consideration of certain pharmacological agents that have a profound effect on the activity of receptors.

The stimulating effect of acetylcholine on receptors is unaffected by atropine and is paralleled by nicotine; the excitant action is blocked by excess acetylcholine or excess nicotine or by curare or hexamethonium. This pattern, and incidentally the concentrations of the drugs required, is very similar to that found in experiments on autonomic ganglia. Activity of a tissue, such as the small intestine, in response to an application of acetylcholine or nicotine could be due to an action on the synapses, but it could equally well be due to stimulation of sensory receptors unless such an action can be excluded by other means. Effects on blood pressure and respiration of rabbits have been produced by local injection of acetylcholine and related substances into the perfused ear; the effects were prevented by hexamethonium (Lembeck, 1957). Since the ear was connected to the body only by the N. auricularis magnus, and the effects were abolished by cocaine, it seemed probable that the responses depended upon excitation of cutaneous sensory pathways.

This similarity of the actions of acetylcholine on sensory receptors and on autonomic synapses does not, however, mean that the physiological role of acetylcholine is the same at the two sites. There is now little doubt that acetylcholine is a synaptic transmitter at autonomic synapses, but there is no evidence that acetylcholine acts as a transmitter at sensory receptors, and the evidence against such a role of acetylcholine at mechano-receptors is quite considerable. The activity of certain mechano-receptors has been shown to be quantitatively unchanged by anticholinesterases and by very large doses of hexamethonium; the biggest concentrations used were ten thousand times those necessary to block the effects of acetylcholine, and this concentration was perfused through the tissue for hours (Diamond, 1955). The only objection to this experiment as evidence against a physiological role of acetylcholine is that the acetylcholine can act not only at the specialized terminal, which is the site of receptor activity (see Gray, 1956), but at another site which is accessible to these drugs; this hypothetical site would have to differ not only from the receptor region, but also from the rest of the axon, which is unaffected by acetylcholine. To this must be added the subsidiary hypothesis that the receptor region is surrounded by a diffusion barrier of a character such that not even one part in ten thousand of the hexamethonium can pass through in several hours.

Finally it must be pointed out that, as in the case of acetylcholine, the effects of adrenaline on sensory receptors could seriously affect the interpretation of experiments on tissue supplied by the autonomic nervous system.

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FUNCTIONAL VASODILATATION IN THE SUBMANDIBULAR SALIVARY GLAND

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When glands and skeletal muscles are active, their blood vessels dilate and the rate of blood flow through them increases. The usefulness of this vascular reaction has long been clear, yet in spite of the close attention paid to it, the mechanisms responsible still remain uncertain. Whereas in skeletal muscle it is generally held that the vasodilatation results from the direct action of locally produced metabolites, in the submandibular salivary gland it has been thought that special vasodilator nerve fibres were responsible. Indeed, the nerve supply to this organ has been regarded by most as an example of a vasodilator innervation characteristic for the parasympathetic division of the autonomic nervous system.

1. Previous Views on Vasodilatation Produced by Chorda Stimulation

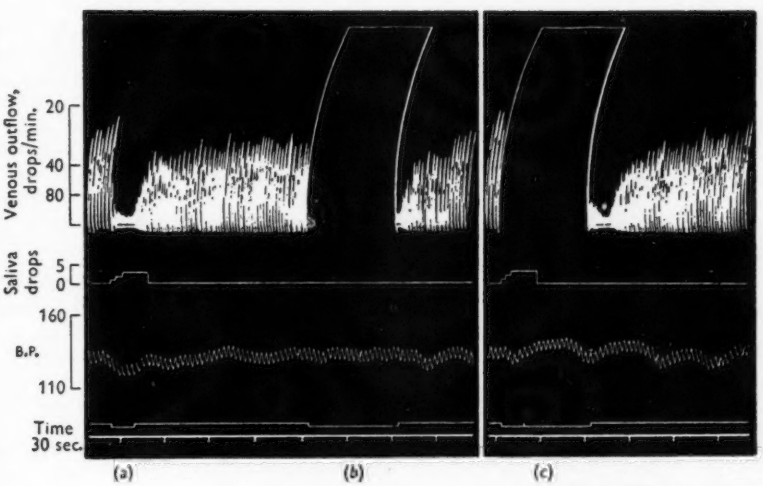
The idea that the nerve to the salivary gland, the chorda tympani, contained vasodilator nerve fibres dates back to the original experiments of Claude Bernard (1858). When he found that stimulation of this nerve produced salivary secretion, increased blood flow and increased heat production, he suggested that each effect was produced by a special set of nerve fibres. Heidenhain's observation (1872) that the vasodilator effect was not abolished by doses of atropine which prevented salivary secretion seemed to support the idea of separate vasodilator nerve fibres.

Nowadays, the postganglionic nerve fibres of the parasympathetic are all thought to be cholinergic. Since the actions of acetylcholine are antagonized by atropine, the relative resistance of the vasodilatation to this drug has been something of a paradox. Dale & Gaddum (1930) suggested that the acetylcholine is released from vasodilator nerve-endings in such great "... intimacy with the receptive mechanism that atropine cannot prevent its access thereto." As early as 1914, however, Barcroft had put forward another point of view. He argued strongly against the idea that the vasodilatation on chorda stimulation was produced by special vasodilator nerve fibres. Having shown that the metabolic activity of the salivary gland increased on nerve stimulation, even when atropine had been given, he accounted for the vasodilatation as the outcome of this metabolic activity, just as had already been suggested in the case of skeletal muscle.

2. A Relatively Stable Substance Resulting from Glandular Activity

When we took up the problem of the vasodilatation in the salivary gland on stimulation of the chorda, we found that it could not be wholly explained by a direct action of acetylcholine; the substance responsible appeared to be relatively stable (Hilton & Lewis, 1955a). This is illustrated in an experiment (see fig. 1), which shows the time-course and extent of the hyperaemia following two 15-sec. periods of

FIG. 1. RECORDS OF VENOUS OUTFLOW FROM SUBMANDIBULAR SALIVARY GLAND, OF SECRETION OF SALIVA, AND OF ARTERIAL BLOOD PRESSURE IN THE CAT



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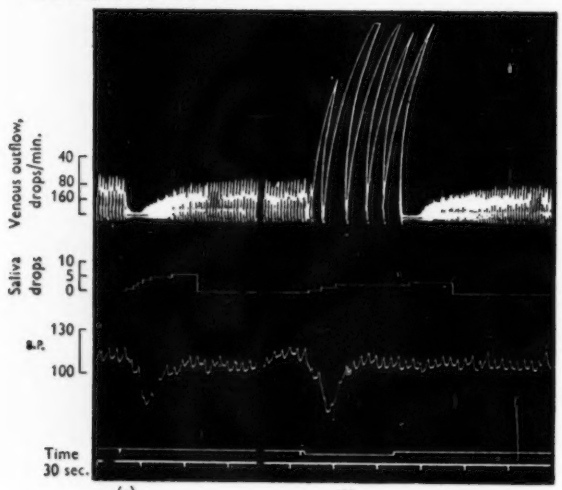
a: effect of stimulation of the chordo-lingual nerve for 15 sec.
b: effect of arterial occlusion for 1 min.
c: effect of chorda stimulation for the first 15 sec. of arterial occlusion for 1 min.
(Venous outflow measured with the Gaddum drop timer.)

chorda stimulation. When the chorda is stimulated at the beginning of a 1-min. period of occlusion of both artery and vein, the hyperaemia on re-establishing the circulation (at *c*) is at least as great as that (at *a*) directly after chorda stimulation. The reactive hyperaemia following a 1-min. period of vascular occlusion alone (at *b*) is by contrast quite small.

It is unlikely that the relatively stable vasodilator substance revealed by these experiments is released by special, non-cholinergic nerve fibres. Evidence for this conclusion was obtained by the use of botulinum toxin which selectively inactivates cholinergic nerve fibres. When the toxin was injected into the gland, the secretory and vasodilator responses to electrical stimulation were affected simultaneously; they diminished and disappeared together. This suggests, in confirmation of Barcroft's argument, that the stable vasodilator substance arises from the gland cells when they are activated by the secretomotor nerve fibres. When the gland is activated by acetylcholine itself, the substance liberated by the secretomotor nerve-endings, the same kind of evidence can be obtained for the production within the gland of the stable vasodilator substance. In the experiment illustrated in fig. 2, acetylcholine is injected arterially into the gland, and the circulation is arrested as soon as the drug has passed through. It is seen that removal of the occlusion clamp 1 minute later is followed by an intense and prolonged vasodilatation. In fact, in this experiment the vasodilatation is greater than that caused by the injection alone.

The direct vasodilator action of acetylcholine would not outlast such a period of circulatory arrest. This is shown in control experiments on skeletal muscle, where acetylcholine exerts such a direct vasodilator action when injected in doses too small to cause muscular contraction. For instance, in

FIG. 2. RECORDS OF VENOUS OUTFLOW FROM SUBMANDIBULAR SALIVARY GLAND, OF SECRETION OF SALIVA, AND OF ARTERIAL BLOOD PRESSURE, IN THE CAT

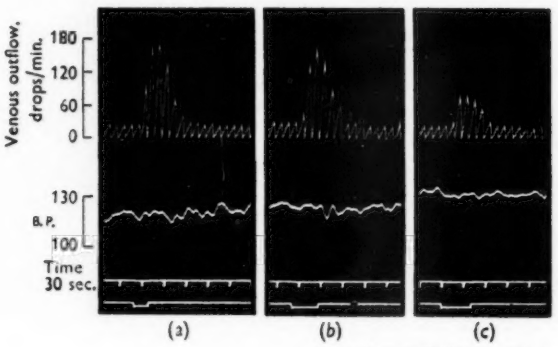


Hilton & Lewis (1955a) by permission of Journal of Physiology

a: effect of arterial injection of 1 μg. acetylcholine

b: effect of arterial injection of 1 μg. acetylcholine followed 5 sec. later by arterial occlusion for 1 min.

FIG. 3. RECORDS OF VENOUS OUTFLOW FROM SUBMANDIBULAR SALIVARY GLAND, AND OF ARTERIAL BLOOD PRESSURE IN THE CAT



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a: effect of stimulation of chordo-lingual nerve for 20 sec.

b: effect of slow arterial injection of 1 ml. perfusate collected previously from the gland during chorda stimulation for 15 sec.

c: effect of slow arterial injection of 1 ml. perfusate collected from the gland at rest

(Venous outflow measured with the Thorp impulse counter.)

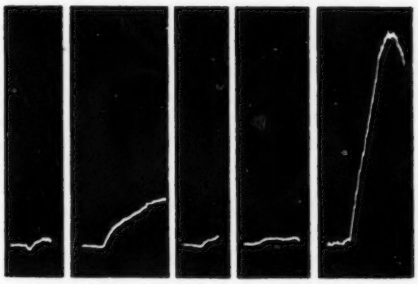
experiments in which the circulation was arrested immediately after an arterial injection of 1 μg. acetylcholine into the cat's gastrocnemius muscle, removal of the occlusion clamp revealed a small, residual effect—no more than the tail-end of the vasodilatation which is seen at the corresponding time after the injection alone.

All these findings provide indirect evidence in support of Barcroft's contention, namely, for the production by the activated gland cells of a stable vasodilator substance, and we therefore looked for such a substance in the venous effluent from the gland. When the experiments were done with the normal blood supply to the gland intact, no such evidence could be obtained. On the other hand, vasodilator activity was found in the effluent during chorda stimulation when the gland was perfused with saline solutions. In these experiments, Locke's solution was perfused through the gland for short periods of 5–10 min., natural circulation being restored in the intervals. After perfusion for 2–3 min., when the effluent was clear of blood, the chorda was stimulated, and the perfusate collected. On re-injection into the gland after its normal blood supply had been re-established, this perfusate produced a pronounced vasodilatation. Perfusate collected from the resting gland produced only a slight hyperaemia, which was accounted for by the dilution of the blood. A typical experiment is shown in fig. 3: at (*a*) the vasodilator effect of chorda stimulation for 20 sec., at (*b*) that of an arterial injection of 1 ml. of perfusate collected during chorda stimulation for 15 sec., and at (*c*) the diluting effect of 1 ml. of perfusate from the resting gland.

3. The Active Principle Released from the Stimulated Gland

The vasodilator activity of perfusate collected during stimulation was not due to acetylcholine or histamine, as it was unaffected by atropine or mepyramine. Nor did the

FIG. 4. EFFECT OF PERFUSATE INCUBATED WITH PLASMA ON GUINEA-PIG ILEUM



Hilton & Lewis (1955b) by permission of Journal of Physiology

Guinea-pig ileum suspended in 15 ml. Tyrode's solution

a: effect of 1 ml. perfusate collected during chorda stimulation for 15 sec.

b: effect of perfusate as in (a), after incubation for 1 min. with 0.2 ml. plasma

c: effect of 0.2 ml. plasma

d: effect of a mixture of 0.2 ml. plasma with a freeze-dried concentrate of perfusate (equivalent to 4 ml.) collected from a resting gland

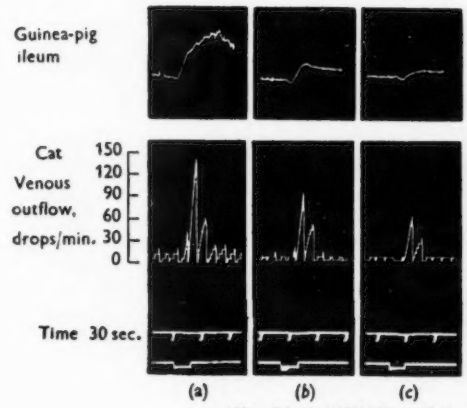
e: effect of a mixture as in (d), but with perfusate collected from the gland during chorda stimulation for 1 min.

perfusate have any action when tested on isolated smooth muscle preparations, such as the guinea-pig ileum. We thought at first that we were dealing with a vasodilator substance having no smooth muscle stimulating activity. However, the real explanation was found to be different. It depended on the fact that different conditions prevail in the experiments in which the perfusate was tested for the two kinds of activity. When being tested for vasodilator activity, the perfusate was injected into the blood going to the gland. On the other hand, when being tested for smooth muscle stimulating activity, the perfusate was simply added to the salt solution in the organ bath. Thus if the vasodilator substance were formed by the action of perfusate on some constituent of the blood, it would not be present in the organ bath, unless the perfusate had been incubated previously with blood. We therefore incubated a mixture of perfusate with blood or plasma and found that this mixture aequired a smooth muscle stimulating activity. This is illustrated in fig. 4. When the same experiment was performed with perfusate concentrated by freeze-drying, the smooth muscle stimulating activity of the mixture was increased.

During these experiments it was found that the smooth muscle stimulating activity depended on the time of incubation. It first increased, but then fell off when incubation was prolonged for more than a few minutes. Such mixtures were also tested for vasodilator activity on the cat's salivary gland and, again, it was found that this activity depended on the time of incubation. Both activities increased at first, then diminished and finally disappeared together. The results of such an experiment are shown in fig. 5. Perfusate collected during chorda stimulation was incubated with plasma, and samples of the mixture were tested after different times of incubation both on the guinea-pig ileum and, by arterial injection, on the cat's salivary gland. The finding that both activities fall off together strongly suggests they are actions of one and the same substance, which is not itself present in perfusate, but is formed and then inactivated when perfusate interacts with plasma proteins.

These results brought to mind some old observations of Secker (1934) and of Feldberg & Guimarais (1935). They found that saliva causes a fall in blood pressure on intravenous injection, but has no smooth muscle stimulating activity, and concluded that saliva contains a depressor substance lacking smooth muscle stimulating activity. The depressor effect is at least partly the result of a peripheral vasodilator action, since we could show that saliva even in high dilution causes vasodilatation when injected into the arterial blood supply to the salivary gland. Were we therefore dealing with a phenomenon similar to that produced by perfusate from the stimulated gland? To examine this possibility, diluted saliva was incubated with plasma and tested on the guinea-pig ileum. The mixture was found to have acquired smooth muscle stimulating activity. A further similarity with the results obtained with incubated perfusate was the finding that the muscle-stimulating activity decreased and finally disappeared as the incubation was prolonged. In the experiment illustrated in fig. 6, the smooth muscle stimulating and the vasodepressor activities of such an incubated mixture of saliva and plasma are seen to diminish and disappear together.

FIG. 5. EFFECT OF PROLONGED INCUBATION OF PERFUSATE WITH PLASMA TESTED ON GUINEA-PIG ILEUM AND SALIVARY GLAND OF CAT



Hilton & Lewis (1955b) by permission of Journal of Physiology

Upper record: guinea-pig ileum suspended in 15 ml. Tyrode's solution

Lower record: venous outflow from submandibular salivary gland of cat, measured with the Thorp impulse counter

a: effect of mixture of plasma and perfusate collected during chorda stimulation, after incubation for 1 min.

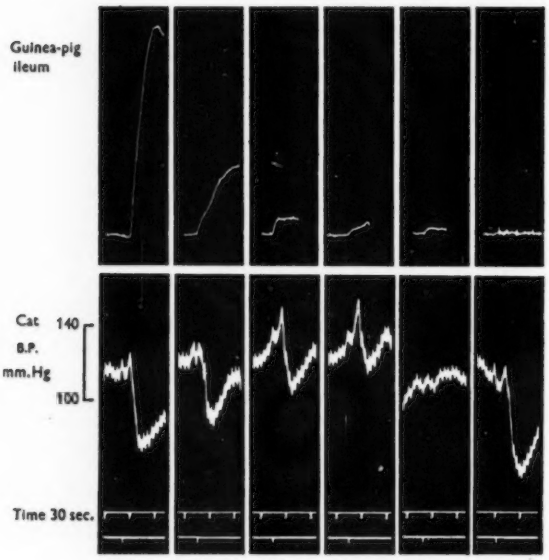
b: effect after incubation for 5 min.

c: effect after incubation for 10 min.

7.5 ml. perfusate were added to 1.5 ml. plasma; 2.4 ml. of the mixture were used for each test on the guinea-pig ileum, and 0.6 ml. for each arterial injection into the salivary gland of the cat.

Time and signal markers refer to lower record only.

FIG. 6. EFFECT OF PROLONGED INCUBATION OF SALIVA WITH PLASMA, TESTED ON GUINEA-PIG ILEUM AND ON BLOOD PRESSURE OF CAT



Hilton & Lewis (1955b) by permission of Journal of Physiology

Upper record: guinea-pig ileum suspended in 15 ml. Tyrode's solution

Lower record: arterial blood pressure of cat

a: effect of mixture of saliva and plasma after incubation for 1 min.

b: effect after incubation for 4 min.

c: effect after incubation for 7 min.

d: effect after incubation for 13 min.

Equal volumes of plasma and 1% saliva were incubated.

0.2 ml. mixture tested on guinea-pig ileum and 1.0 ml. on blood pressure of cat.

e: effect of plasma on guinea-pig ileum (0.1 ml.) and on blood pressure of cat (0.5 ml.)

f: effect of 1% saliva on guinea-pig ileum (0.1 ml.) and on blood pressure of cat (0.5 ml.)

Time and signal markers refer to lower record only.

4. Similarity between Bradykinin and the Substance Formed by Perfusate and Saliva

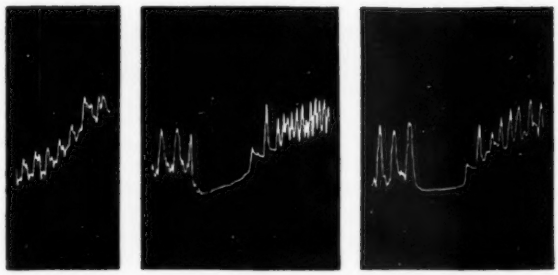
The formation of the vasodilator and smooth muscle stimulating substance when active perfusate or saliva is incubated with plasma, and the disappearance of activity on prolonged incubation, resemble the formation and inactivation of a known vasodilator and smooth muscle stimulating polypeptide which has been called bradykinin or kallidin. The name bradykinin was given by Rocha e Silva, Beraldo & Rosenfeld (1949) to a slowly acting, smooth muscle stimulating polypeptide produced on incubation of snake venom or trypsin with serum, plasma or fractionated plasma globulin; and the name kallidin was coined by Frey, Kraut & Werle (1950) for a slowly acting, smooth muscle stimulating substance, also a polypeptide, produced by the action of tissue extracts on plasma proteins. The active agent in the tissue extracts is thought to be a proteolytic enzyme and has been called kallikrein. Bradykinin and kallidin are either the same substance or closely related polypeptides. It is interesting that originally kallikrein had also been thought of as a vasodepressor substance in its own right.

The experiments described below show that the substance formed by active perfusate or saliva is bradykinin itself or a closely related polypeptide.

A powder containing the active substance was obtained from an incubated mixture of saliva and globulin solution, according to the method used by Rocha e Silva *et al.* (1949) for the preparation of bradykinin. The active substance in this powder was found to have the same pharmacological properties as bradykinin, a sample of which was kindly given to us by Dr Rocha e Silva. Solutions of both, when incubated with plasma or globulin solutions, diminished in activity, with the same time-course. Both solutions caused contraction not only of the guinea-pig ileum but also of the rabbit intestine, rat colon and rat uterus. The latency, rate of development and duration of the contractions were the same for both. Further, on the rabbit intestine and rat colon both solutions produced an initial relaxation and inhibition of the small rhythmic movements before the stimulating effect appeared. In this particular respect, the effect of these solutions differed from that of another smooth muscle stimulating polypeptide, substance P. Figure 7 shows a comparison of the action on the rat colon of substance P with bradykinin and with the powder prepared from saliva and a globulin solution. Substance P lacks the initial inhibitory action.

The results of these tests, although very suggestive, are not sufficient to identify the vasodilator and smooth muscle stimulating substance with bradykinin. Greater certainty can be achieved by comparing the effects of the two substances quantitatively on different experimental preparations. When the effects of bradykinin and the powder prepared from saliva and globulin solution on the guinea-pig ileum, rat uterus and rat colon were compared in this way, there was good agree-

FIG. 7. COMPARISON OF ACTION ON RAT COLON, OF SUBSTANCE P, OF A POWDER PREPARED FROM SALIVA AND GLOBULIN SOLUTION, AND OF BRADYKININ



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Ascending colon of the rat suspended in 15 ml. de Jalon's solution.

a: effect of 400 μg. substance P

b: effect of 30 mg. of powder

c: effect of 800 μg. bradykinin

TABLE I. AMOUNT OF STANDARD BRADYKININ AND OF SUBSTANCE P EQUI-ACTIVE WITH 1 mg. OF POWDER

	Bradykinin (μg.)	Substance P (μg.)
Guinea-pig ileum	20	57.2
Rat uterus	27.8	8,000
Rat colon	28.8	11.6
Cat blood-pressure	30	—
Vasodilatation in cat salivary gland	20	—

ment. This held also when the comparison was extended to their vascular effects in the cat, using their vasodilator action in the salivary gland and vasodepressor action on intravenous injection. The results of these quantitative comparisons are shown in Table I.

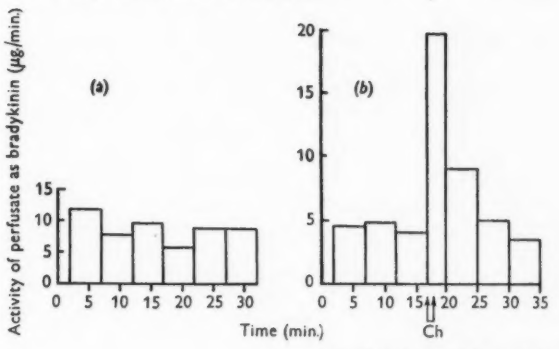
The small differences in activity between bradykinin and the powder prepared from saliva and globulin are within the limits of experimental error, and contrast strikingly with the results of a comparison of the effects of the powder with those of substance P on the three smooth muscle preparations, which are also shown in the table.

Finally, some of the physico-chemical properties of bradykinin were compared with those of the active substance in the powder. Both showed the same solubility in organic solvents and the same resistance to heat, changes in pH and proteolytic enzymes (Hilton & Lewis, 1955b).

5. Quantitative Estimation of the Release of Bradykinin-Forming Enzyme

The results described so far indicate that the activity of perfusate collected during chorda stimulation, and of saliva, are due to the presence in these fluids of an enzyme or activator which forms a polypeptide like bradykinin when in contact with plasma globulins. An attempt was therefore made to estimate this enzyme or activator quantitatively in perfusates collected from the salivary gland (Hilton & Lewis, 1956a). The method used was as follows. A standard procedure was adopted for incubating perfusate with globulin solution for 1 min. at 30° C. The mixture was then assayed against a standard preparation of bradykinin on the rat uterus. This tissue is so sensitive that 1–3 μg. of the bradykinin powder used were sufficient to contract the uterus when suspended in a 15-ml. bath. With this technique, it was found that some bradykinin-forming enzyme escaped from the perfused gland even at rest. The amount remained fairly steady over quite a long period of time, as was shown in experiments in which perfusion was maintained for over 30 min. (fig. 8a). On the other hand, if the chorda was stimulated for 1 min. after 10 min. of perfusion, the enzyme activity immediately increased 2.5–8.5 times, and then slowly returned to the resting value. In the experiment shown in fig. 8b, the increase is fourfold. A similar increase was obtained when, instead of stimulating the chorda, the perfused gland was activated by an arterial injection of acetylcholine. In fact, a dose of acetylcholine which has a vasodilator action of duration and extent similar to chorda stimulation for 1 min. produced the same release of enzyme, as judged by the close similarity of the histogram profiles. The injected acetylcholine acts directly on the gland cells and not on the ganglion cells in the hilus of the gland, since it gives rise to the same increase in enzyme release after a previous injection of a dose of hexamethonium sufficient to prevent stimulation of the postganglionic nerve fibres to the gland.

FIG. 8. OUTPUT OF BRADYKININ-FORMING ENZYME FROM THE SUBMANDIBULAR GLAND, PERFUSED FOR 35 MIN., IN TWO CATS



Hilton & Lewis (1956a) by permission of Journal of Physiology

a: output from resting gland

b: output from gland activated by chorda stimulation for 1 min. (at Ch)

In each experiment the gland was perfused for 2 min., then perfusate was collected in 5-min. periods, except for the 3-min. sample collected during, and in the 2 min. following, chorda stimulation.

6. Site of Formation and Action of Bradykinin under Natural Conditions

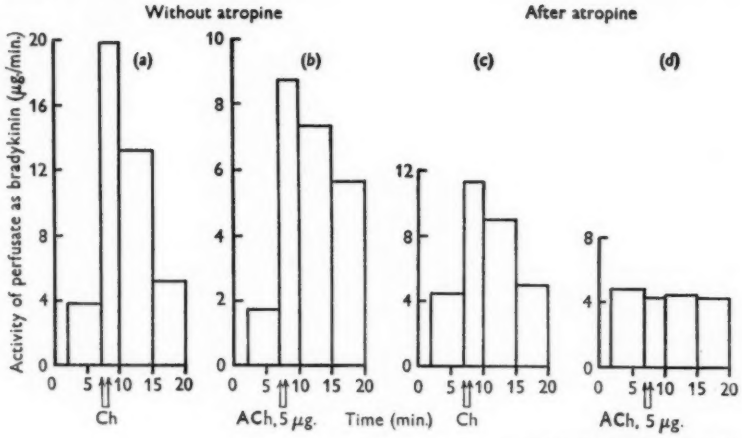
We have seen that the active material, which is released from the gland cells on chorda stimulation and appears in the perfusate, does not itself have a vasodilator action. It has to come into contact with plasma proteins to produce the vasodilator substance, which is a polypeptide and apparently identical with bradykinin. Under normal physiological conditions the formation of this substance probably occurs in the interstitial fluid of the gland. There are good grounds for assuming that interstitial fluid contains the substrate for the enzyme released from the gland cells during activity. Chemical and electrophoretic analyses have shown that mammalian lymph, which is probably identical in composition with interstitial fluid, contains all the protein fractions of plasma (Yoffey & Courtice, 1956). The actual amounts are somewhat smaller; in cervical duct lymph, for example, the values are one-half to one-third those of plasma.

Bradykinin, once formed in the interstitial fluid, is probably eliminated via the lymph, as indicated by the following observations. It has already been mentioned that the vasodilator substance formed in the activated gland could be retained when both artery and vein were occluded. In further experiments it was shown that the substance was not retained by venous occlusion alone, which keeps the blood in the gland, but increases the lymph flow from it. On the other hand, when the gland was carefully separated from the surrounding tissues to prevent any lymph drainage, venous occlusion alone often led to full retention of the vasodilator substance. The effect was as great as that obtained after

occlusion of both artery and vein in the gland with undisturbed lymph drainage (Hilton & Lewis, 1955a).

Although the enzyme can be detected in the effluent from a perfused gland, it is unlikely that it ordinarily passes from the interstitial fluid into the capillaries. It is only when the gland is perfused with artificial saline solutions that the capillaries become sufficiently permeable to allow the enzyme to pass. Indeed, under these conditions some leakage of enzyme occurs even when the gland is at rest. It seems reasonable to assume that normally most, if not all, of the bradykinin formed in the interstitial fluid will be prevented by the limited permeability of the capillaries from reaching the blood in them. It will produce its vasodilator effect locally and be eliminated by the lymph, either by inactivation or in the course of lymph drainage. If any should reach the blood stream, it will be rapidly inactivated there too. It is therefore not surprising that it could not be detected in venous blood samples.

FIG. 9. OUTPUT OF BRADYKININ-FORMING ENZYME FROM THE SUBMANDIBULAR SALIVARY GLAND, PERFUSED FOR 20 MIN., IN FOUR CATS



Hilton & Lewis (1956a) by permission of Journal of Physiology

a: gland activated by chorda stimulation for 1 min. (at Ch); experiment performed without atropine

b: gland activated by infusion of 5 μg. acetylcholine; experiment performed without atropine

c: gland activated by chorda stimulation for 1 min. (at Ch); experiment performed after intravenous injection of atropine, 0.5 mg./kg.

d: gland activated by infusion of 5 μg. acetylcholine; experiment performed after intravenous injection of atropine, 0.5 mg./kg.

In each experiment the gland was perfused for 2 min., then perfusate was collected in 5-min. periods, except for the 3-min. samples collected during, and in the 2 min. following, activation.

7. Chorda Stimulation after Atropine

We have studied the enzyme-release in perfused glands after an injection of atropine, and found that, whereas the output is no longer increased by intra-arterial acetylcholine, it is still increased by chorda stimulation. This is shown by the experiments illustrated in fig. 9. The increase in enzyme-release was smaller than normal, being 2–2.5 times the resting level. This recalls the observations of Barcroft (1914) who measured oxygen consumption, and those of Anrep & Cannan (1922) who studied glucose utilization. Both found an increase in metabolic activity on chorda stimulation, even after atropine, though the increase was smaller. Atropine thus only reduces, but does not abolish, the metabolic effect of chorda stimulation in the salivary gland, and it now looks as though this remaining effect is responsible for increased bradykinin formation and hence for the atropine-resistant vasodilatation.

This explanation implies that the secretomotor nerve fibres of the chorda are in some way resistant to the action of atropine. Overt secretion is prevented, but some of the biochemical processes associated with glandular activity are still stimulated. Since injected acetylcholine has none of these effects after atropine, either these nerve fibres release some other transmitter substance in addition to acetylcholine, or the acetylcholine is released in such close proximity to the receptor structures that atropine cannot completely block its access to them. This view differs from that of Dale & Gaddum (1930) in so far as they assumed the acetylcholine to be released in intimate relationship with the smooth muscle of the vessel wall. According to our conclusion, the acetylcholine would be released in this relationship with the gland cells, and the atropine-resistant vasodilatation would be the result of partial activation of these cells and the subsequent formation of bradykinin.

8. Bradykinin Formation Caused by Sympathetic Stimulation and Sympathomimetic Amines

According to our view, the hyperaemia occurring in the salivary gland on chorda stimulation, whether or not the gland is treated with atropine, must be regarded mainly as a functional hyperaemia, i.e., the vasodilatation is secondary to activity of the glandular tissue. There is also evidence for such a functional hyperaemia when the glands are activated by sympathetic stimulation or by injection of sympathomimetic amines. However, such evidence has so far been obtained only in cats.

In the cat, stimulation of the cervical sympathetic trunk leads to salivary secretion, and, though the blood flow through the gland can be brought almost to a stand-still while the stimulus is applied, the cessation of the stimulus is followed by a vasodilatation which may be large and prolonged. Similar secretory and vascular effects are produced by arterial injections of noradrenaline and adrenaline.

Again, Barcroft brought forward evidence in favour of the view that the adrenaline vasodilatation is the result of metabolic activity. He showed (Barcroft & Piper, 1912; Barcroft, 1914) that, after an intravenous injection of adrenaline, the vasodilatation which follows the initial constriction shows the same time-course as the increase of oxygen uptake by the gland. Moreover, anti-adrenaline drugs, in doses which

abolish the secretory effect of cervical sympathetic stimulation, noradrenaline and adrenaline, reverse the vasoconstrictor effect and abolish the after-dilatation (Barcroft, 1914; Hilton & Lewis, 1956a). If the after-dilatation were a direct effect of the sympathomimetic amines, we should expect it to be resistant to anti-adrenaline drugs, because all vasodilator responses to these amines, for instance in skeletal muscle, persist unchanged after anti-adrenaline compounds. However, the after-dilatation is abolished by these compounds, and this fact is readily explained by the hypothesis that the prolonged after-dilatation is due to activation of the gland.

The effect of noradrenaline and adrenaline on the output of bradykinin-forming enzyme from the perfused gland was studied. These amines caused an increase in the output at least as great as that produced by chorda stimulation (Hilton & Lewis, 1956a). Thus the vasodilatation following sympathetic stimulation can also be explained by bradykinin-formation resulting from glandular activity, and must be looked upon as another example of functional hyperaemia.

9. Vasodilator Effect of Chorda Stimulation in the Tongue

Heidenhain (1883) showed that stimulation of the chorda tympani caused vasodilatation not only in the submandibular salivary gland, but also in the tongue; and it has since been accepted that this vascular response is due to special vasodilator nerve fibres similar to those thought to innervate the blood vessels of the salivary gland (Machol & Schilf, 1928; Erici & Uvnäs, 1952). A particular point of similarity is that atropine reduces but does not abolish the vasodilatation in the tongue. Since there are little islands of glandular tissue embedded in the tongue, whose circulation is probably shared in common with that of the whole organ, it was natural to consider the bradykinin-forming mechanism as possibly being responsible for the vasodilatation in this organ also.

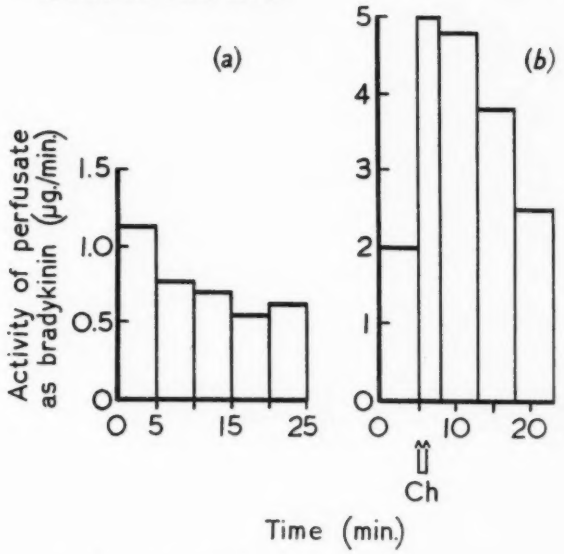
When the effluent from the perfused tongue was examined for bradykinin-forming activity, small amounts were found in the absence of nerve stimulation and this activity increased on stimulation of the chorda (Hilton & Lewis, 1956b). The activity of "resting" perfusate was sometimes only just measurable, and the increase after chorda stimulation for 1 min. was only 2.5–3.5 times the resting level. Thus, the amounts were smaller than the corresponding values in the salivary gland experiments. Typical experiments on the tongue are illustrated in fig. 10. It is not surprising that the values are relatively small, because of the small proportion of glandular elements in the tongue as compared with the muscle which forms the bulk of the organ. Acetylcholine injections also produced an increase, comparable with that of chorda stimulation. That the enzyme does not come from the striated muscle fibres is shown by the finding that stimulation of the hypoglossal, the motor nerve to the tongue, does not produce any increase in enzyme output.

The fact that the atropine-resistant vasodilatation in the tongue is so intense may well be explained by the opening up by bradykinin of the arteriovenous anastomoses in this organ.

10. Bradykinin-Forming Enzyme in Glandular Secretions

The presence of large amounts of the bradykinin-forming enzyme in saliva invites some speculation about its significance in this secretion. A remarkable feature of the anatomy of the

FIG. 10. OUTPUT OF BRADYKININ-FORMING ENZYME FROM THE TONGUE, PERFUSED FOR 25 MIN. IN TWO CATS



a: output from unstimulated tongue

b: effect of chordo-lingual stimulation for 1 min. (at Ch)

In each experiment perfusate was collected in 5-min. periods, except for the 3-min. sample collected during, and in the 2 min. following, chordo-lingual stimulation.

submandibular salivary gland is the rich vascular supply to the duct system (Kowalewsky, 1885). Each duct appears to have its own artery and vein, with a well-developed capillary network penetrating the duct wall. We may therefore consider the possibility that materials may be transferred directly across the duct wall and, indeed, Burgen (personal communication) has recently presented evidence that radioactive plasma albumin can act as a source for saliva protein probably formed in the duct cells. Since this implies that plasma proteins can enter the duct cells, it is possible to envisage the bradykinin-forming enzyme travelling in the other direction, i.e., from the lumen of the duct outwards to the interstitial fluid. It has been shown that, if the hydrostatic pressure in the duct system is artificially raised, a marked vasodilatation develops in the gland (Hilton & Lewis, 1955b), and this may be a reflection of the ease with which the enzyme can pass across the duct wall. The implication of this suggestion is that the enzyme does not only pass directly from the activated gland cells into the interstitial fluid, but that, being present in saliva, it can also diffuse through the duct wall. The fact that the ducts are supplied with an abundance of small arteries which run alongside and close to them would then ensure a pronounced increase in blood flow through the secreting gland.

Bradykinin-forming enzyme is present not only in saliva, but in other glandular secretions also. Where it occurs, it shows that these glands possess the bradykinin-forming mechanism, which raises the possibility that functional vasodilatation in these glands is also produced by bradykinin. For example, pancreatic juice produces, on incubation with

plasma, a polypeptide (called kallidin) which bears a striking similarity to bradykinin and is probably identical with it (Werle & Berek, 1950; Werle, Kehl & Koebke, 1950). Not only does human forearm sweat contain a bradykinin-forming enzyme, but it has now been shown that the bradykinin content of the subcutaneous tissue of the forearm increases when the subject is heated, and it has been concluded that this mechanism contributes to the vasodilatation which develops in the skin at the same time (Fox & Hilton, 1956, 1957). It is interesting that a similar enzyme is also present in urine (Frey *et al.* 1950; Beraldo, 1952; Beraldo, Feldberg & Hilton, 1956); this indicates perhaps that the mechanism may play a part in the regulation of renal blood flow.

11. Conclusion

In conclusion, therefore, the bradykinin-forming mechanism which plays an important role in the production of functional vasodilatation in the submandibular salivary gland may be a fundamental means of adjustment of local circulation to local needs in many organs and tissues. This conclusion applies to some pathological as well as physiological reactions. Release of intracellular enzymes is known to occur following cell damage produced by burns of the skin (Beloff & Peters, 1945); but more trivial forms of skin trauma, which lead to the development of local vascular reactions only, may act in this way also. It was always known that the " H-substance " of Sir Thomas Lewis, which is produced under these conditions in the human skin, was not really a single substance. Lewis (1927) and Krogh (1929) postulated the existence of an H-colloid which was eliminated slowly, and primarily in the lymph. The necessary role could be played by bradykinin, or the enzyme producing it. This suggestion is strengthened by the finding that the pain-producing substance which develops in the fluid aspirated from a blister in the human skin and in inflammatory exudates is closely related to bradykinin, if not bradykinin itself (Armstrong, Keele, Jepson & Stewart, 1954; Armstrong, Jepson, Keele & Stewart, 1957).

Plasmin (or fibrinolysin), a proteolytic enzyme of blood, also forms a bradykinin-like substance on incubation with plasma globulins (Beraldo, 1950; Lewis & Work, 1957). Since plasmin has been shown to be activated by streptokinase and staphylokinase (Milstone, 1941; Christensen, 1945) and by activators from tissue cells (Astrup & Permin, 1948; Astrup, 1951), this bradykinin-forming system in plasma may be involved in the production of inflammatory reactions. Indeed, this may be the system with which Armstrong *et al.* (1957) were concerned. Following the demonstration of increased fibrinolytic activity in the plasma of dogs during anaphylactic and peptone shock (Rocha e Silva, Andrade & Teixeira, 1946), Beraldo (1950) has gone further, to show that the plasma, in these conditions, often contains bradykinin-like activity. This raises the interesting possibility that bradykinin plays a contributory role in the production of the peripheral vascular collapse which is the essential feature of the shock syndrome. Thus, the bradykinin-forming mechanism may be involved in many physiological and pathological phenomena, though particularly in the adjustment of local vascular control.

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SWEATING IN RELATION TO SYMPATHETIC INNERVATION

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Two types of sweat glands are histologically distinguishable, viz. the eccrine and the apocrine glands (Schiefferdecker, 1917). The eccrine glands, being the more accessible, have been the more extensively studied. They occur abundantly over most of the human body-surface, and in the pads of the feet in the cat. Apocrine glands are found, together with eccrine glands, in scattered areas of the human skin, notably in the axilla and, without eccrine admixture, over the whole sweating area in the horse.

The distinction between the two types has until recently been regarded rather as of histological interest than as signifying any sharp differentiation in their functioning, though Kuno (1956) has for many years stressed a fundamental physiological difference, and has quite recently even considered a subdivision of the apocrine glands into the two groups of " osmidrotic " or odour-producing glands and " anosmidrotic " glands, which do not produce odour. There is, in fact, now a convergence of evidence that eccrine and apocrine sweat glands show considerable differences in their physiological properties and functions, and that generalizations from one type to another are likely to be misleading. Even within each type there is not complete uniformity. For instance, the eccrine glands over the main surface of the human body are mainly brought into action in hot surroundings, but those of the palms and soles respond to mental rather than to thermal stress. Also, the apocrine glands of the horse, as regards the amounts of secretion which they can produce, the presence of glycogen in their secreting cells (Evans, Nisbet & Ross, 1957) and their relations to temperature regulation, resemble the eccrine glands rather than the apocrine glands of the human subject.

1. Secretory Nerves to Sweat Glands

The first instance of any secretory nerve to be discovered was that of the chorda tympani to the submandibular gland (Ludwig, 1851). The second example was discovered incidentally by Goltz (1875), who noticed that stimulation of the sciatic nerve in the cat caused first pallor and then flushing and sweating of the pads of the hind feet. It was soon shown that the nerve fibres concerned were sympathetic (Kendall & Luchsinger, 1876; Ostroumow, 1876) and that their action was not primarily dependent upon alterations in the vascularity of the skin, since, like that of the chorda, it was checked by atropine but not by transitory stoppage of the blood supply. When the discovery of other secretory nerves (e.g., to stomach and pancreas) followed, the generalization that all secretory activity was aroused by the action of nerves was tacit and understandable, but was shaken by the discovery of pancreatic secretin (Bayliss & Starling, 1902), which initiated the general conception of humoral action and led to the coining of the word " hormone ".

No efferent secretory nerve fibres, other than sympathetic, have been satisfactorily demonstrated to the eccrine glands, the response of which to sympathetic nerve stimulation is prompt (a matter of seconds) and copious. The anatomy of the sympathetic supply to the skin was worked out in detail in the cat by following the course of the sudomotor fibres to the pads, as indicated by the results of stimulation of nerves, spinal roots and grey rami, and by use of the nicotine method (Langley, 1891); and of the pilomotor fibres to the skin generally in cats and monkeys (Langley & Sherrington, 1891; Langley, 1894). Subsequent clinical studies have shown that in man the sympathetic supply to the skin, as judged by the distribution of vasomotor, sudomotor and pilomotor fibres, is—as forecast by Langley (1894)—in general anatomical accordance with the findings in the cat. It must be stressed that, prior to Schiefferdecker's work in 1917, all sweat glands were supposed to be of the same type, and it came to be assumed that results obtained on any sweat glands could be considered as applicable to all; from which some confusion has arisen.

2. Response of Eccrine Glands to Chemical Agents

The failure of the glands of the cat's pads to respond to systemically administered adrenaline by sweating was noted by Langley (1901); yet the arrectores pilorum do react to both adrenaline and noradrenaline. The eccrine glands in general have long been known to respond to parasympathomimetic stimulants such as pilocarpine, acetylcholine or carbachol, whether administered systemically or intradermally. Moreover, the concentration threshold for acetylcholine on intradermal injection in man, normally about 10^{-5}, is lowered to one-hundredth, i.e., to 10^{-7}, by previous injection of an anticholinesterase, e.g., tetraethyl pyrophosphate (0.1 ml.) (Chalmers & Keele, 1952).

It was thus inferred that, whereas the arrectores pilorum are sympathetic and adrenergic, the eccrine sweat glands are sympathetic and cholinergic. That the sudomotor transmitter for these glands is, in fact, acetylcholine was shown by Dale & Feldberg (1934), who demonstrated the appearance of acetylcholine in the venous blood from the cat's hind foot when the abdominal sympathetic was stimulated, and further showed that, when the pads were tied off, this release of acetylcholine into the blood no longer took place.

The results of intradermal injections into skin provided with eccrine glands are anomalous, however, in that sweating results not only from the injection of parasympathomimetic

substances, but also, together with pilo-erection and vasoconstriction, from the injection of adrenaline or noradrenaline (Kisin, 1948; Haimovici, 1948; Sonnenschein, 1949; Sonnenschein, Kobrin & Grossman, 1949; Janowitz & Grossman, 1950; Chalmers & Keele, 1951, 1952). It was shown by Mellinkoff & Sonnenschein (1954) that the same individual glands secreted in response to heat, to intradermal adrenaline, and to intradermal acetylcholine. The response to intradermal adrenaline led to the suggestion (Haimovici, 1950) that there might be a double innervation of the sweat glands, one cholinergic and one adrenergic, since the response to adrenaline, but not that to Mecholyl, was abolished by the anti-adrenaline agent Dibenamine, while atropine abolished the response to Mecholyl but not to adrenaline. However, since thermal or emotional (palm) sweating is inhibited by atropine but not by anti-adrenaline agents (Priscol) (Chalmers & Keele, 1951), it seems clear that the nerve supply to the glands is solely cholinergic. The phenomenon of the response to intradermal adrenaline is at present unexplained, and though it would at first sight seem that a likely explanation is that the adrenaline, by contracting the myoepithelial cells, merely expresses preformed sweat, it is pointed out by Sonnenschein, Kobrin, Janowitz & Grossman (1951) that the amount and duration of the secretion are much too great for this to be the explanation.

3. Response of Apocrine Glands to Chemical Agents

a. In the Horse

In a text-book of veterinary therapeutics, Kaufmann (1910), apparently unaware of its significance, made the casual statement that adrenaline given subcutaneously to horses caused

> . . . l'apparition d'une sudation locale qui offre un caractère tout particulier; elle se localise exclusivement le long des vaisseaux lymphatiques et en dessine le trajet . . . Cette sudation apparaît environ une heure après l'injection et dure plusieurs heures.

The observation remained unnoticed, and the apparent paradox of the sudomotor action of adrenaline in the horse was independently rediscovered by Fröhner (1915) and by Muto (1916), and was confirmed by Habersang (1921), Langley & Bennett (1923), Bacq (1932) and Pitre (1945). All these workers gave the adrenaline by the subcutaneous or intravenous routes, and usually in large doses (0.12–0.27 mg./kg.).

The administration of adrenaline by intradermal injection (Evans & Smith, 1956) demonstrates how accurate Kaufmann's observation was. Lymphatic spread of adrenaline vasoconstriction following intradermal injection is well known in man: the spread is branching and irregular; in the horse the results are much more striking—long, narrow and almost unbranched tracks of sweating passing often for 30–50 cm. from the site of injection towards deeper lymphatics. The threshold dose is about 0.1 μg. Adrenaline given intravenously (0.5–1.0 μg./kg.) causes general sweating, about equal (if the skin is not too cold) to that resulting from a canter of a mile[1] or two; thus, the sweat glands are evidently very sensitive to it.

Noradrenaline given intradermally causes little and delayed, or no, sweating, but results in pilo-erection along similar tracks (Evans & Smith, 1956), or, when given intravenously, in doses up to 12 μg./kg., may cause general pilo-erection, but again no sweating.

[1] 1 mile = 1.6 km.—Ed.

This action of adrenaline is not inhibited by atropine, nor by any of the seven adrenaline antagonists which have been tested, whether administered systemically, intradermally by injection, or by iontophoresis; but the pilo-erection caused by noradrenaline is inhibited by Dibenamine. So far as the evidence goes, therefore, it would seem that in the horse the innervation of the pilo-erectors is of the usual adrenergic type, and is operated by noradrenaline, but that the sweat glands are adrenergic, and are operated by adrenaline.

Towards parasympathomimetic substances the horse sweat glands appear at first sight to present the converse of human eccrine glands, for, though adrenergic, they respond to intradermal injections of these drugs (e.g. acetylcholine, carbachol) by free sweating which is inhibited by atropine and augmented by anticholinesterases. On intravenous injection of small doses of parasympathomimetic substances, there is no sweating response, but after large doses of carbachol (10 μg./kg.) or smaller doses of acetylcholine (2 μg./kg.) when preceded by an anticholinesterase, general free sweating ensues. This can perhaps be attributed to release of adrenaline from the adrenals (Evans, Smith & Weil-Malherbe, 1956) rather than to a direct action on the sweat glands, as it is known that adrenaline release is facilitated by anticholinesterases and that the preganglionic splanchnic fibres are cholinergic (Feldberg, Minz & Tsudzimura, 1934).

In cattle, the sweat glands are also adrenergic (Taneja, 1956).

b. In Man

The careful studies of Shelley and his associates (Shelley, 1951; Shelley & Hurley, 1952, 1953; Hurley & Shelley, 1954) have thrown much light on the functioning of the apocrine glands of the human axilla. Apart from the great difference in the amounts of secretion produced, these seem broadly to resemble the general sweat glands of the horse. In the axilla, however, both eccrine and apocrine glands occur; the former capable of secreting copiously, showing thermal sweating, and responding to intradermal injection of parasympathomimetic drugs, both effects being paralysed by atropine. Cholinesterase can be histologically demonstrated about the tubular parts of the eccrine glands, but the apocrine glands of the axilla show none (Hurley, Shelley & Koelle, 1953).

After atropine administration, the axillary apocrine glands can be studied alone. They secrete scantily and intermittently, show emotional sweating (severe pain) but not thermal sweating, respond to sympathomimetic but not to parasympathomimetic drugs, and are not paralysed by atropine. It is well known that eccrine sweating in response to heat or to mental stress is abolished by sympathectomy, but apocrine emotional sweating in the axilla is stated not to be abolished by sympathectomy (Shelley & Hurley, 1952).

4. The Sympathetic, and Sweating of the Face and Neck

The study of the innervation of the sweat glands of the face and neck presented difficulties to the older physiologists who were engaged in following the sympathetic supply to the skin, because of the absence or inactivity of sweat glands on the face and neck in most animals. Special species therefore had to be studied.

a. In the Pig

The pig's snout-disk (Rüsselscheibe), which has sweat glands, was found by Luchsinger (1880) and Nawrocki (1880)

to be supplied with sympathetic fibres from the second to fourth spinal roots, which passed via the cervical sympathetic to the infra-orbital branch of the fifth nerve.

I have confirmed the sympathetic innervation of the sweat glands of the pig's snout, and found the action to be cholinergic, and arrested by atropine (0.1 mg./kg.). The glands occur in very large clusters, especially near the rim of the disk. On stimulation of the peripheral end of the cut cervical sympathetic trunk, large drops of sweat appear in a few seconds on the rim and disk on that side, which also turns paler. Section of the cervical sympathetic arrests thermal sweating of the decentralized side of the disk. Acetylcholine or adrenaline (10 μg./kg. intravenously) or carbachol (50–100 μg./kg. intravenously) causes sweating, but since that by adrenaline cannot be repeated a second time soon after a first, it is probably due to an action on the myoepithelial cells, though admittedly the amount of sweat produced by a first injection seems rather large for that. It can be concluded that the general behaviour is that of eccrine glands.

b. In the Sheep

Stimulation of the cervical sympathetic causes slight sweating, so does the intradermal injection of adrenaline; but, since a second stimulation or injection does not have any effect, it would appear that the action is only upon the myoepithelial cells, causing the expression of a small amount of preformed secretion. As carbachol produces no sweating, the cause of the formation of secretion remains uncertain.

c. In the Horse

When the earlier experiments were in progress, the horse, which sweats over the whole body, seemed to be the animal of choice, and the distinction between eccrine and apocrine glands was not then known. The results of these experiments were, however, complicated by a still earlier observation. Dupuy (1816) had noticed, incidentally, that after section of the vago-sympathetic trunk, or excision of the guttural (anterior cervical) ganglion, spontaneous sweating of the head and upper neck on the same side set in after a variable interval of time, and continued for many hours. Claude Bernard (1853) repeated the observation, but seems not to have been surprised by the sweating, which he regarded as incidental to the increase of "calorification" in the areas, sweat nerves being then unknown. After sudomotor nerves had been discovered, however, there arose from this observation the notion of sweat-inhibitory nerves (Vulpian, 1878), which, after some controversy, was ultimately rejected, and, together with the facts from which it originated, conveniently forgotten. The Dupuy phenomenon has been confirmed by several observers (Mayer, 1826, on donkey; Luchsinger, 1880; Muto, 1916; Evans & Smith, 1956; Bell & Evans, 1956). Immediately after the section of the sympathetic or of the vago-sympathetic trunk, the skin temperature of the decentralized area is raised and, though it slowly returns, remains slightly raised for several weeks. The denervated area sweats more readily than the rest of the body on exercise, or in response to adrenaline, locally or systemically, for several weeks or months. Dupuy's experiments were made before the introduction of anaesthesia, and it is probable that adrenaline release was a factor which contributed to making the onset, localization and amount of the sweating so obvious as to have forced itself upon his notice. The sweating is unilateral, and is localized to the area which has been decentralized, and, despite Luchsinger's assertion to the contrary, does occur in animals under deep chloral anaesthesia (Bell & Evans, 1956), though then more scanty. The time at which sweating begins after sympathetic section depends on the vascularity of the skin, and thus on skin temperature, and if the skin is too cold it may not appear at all. For that matter, the sweat response to intravenous adrenaline is also much reduced in cold surroundings.

The crucial test of secretory function must be the result of stimulation of the oral end of the severed nerve. If there is any parallel with the functions of the sympathetic in relation to the eccrine glands, then stimulation should cause immediate secretion of sweat. This it does not do; vasoconstriction and some pilo-erection follow, but there is no sweating at all so long as the stimulus is continued. Not only so, but spontaneous sweating established after sympathetic section is arrested on stimulation of the cervical sympathetic. Further, the stimulation prevents or greatly reduces the sweating following intravenous adrenaline injection, no doubt because of the local vasoconstriction which it produces (Bell & Evans, 1956).

It seems from what has been said that in the horse the sweat glands, at least in the head and neck, do not receive secretory fibres from the sympathetic, as is the case in the pig, and that the horse was badly chosen as a species from which to deduce the innervation in man, where, according to the results of operations for sympathectomy, there are sudomotor as well as pilomotor sympathetic fibres to the head and neck, as to the rest of the body.

The inference which can be made from the above experiments is that the apocrine glands of the horse are not operated by sympathetic nerves, or indeed, by any nerves at all, since no other source of innervation has been proved to exist, nor has it been suggested. Luchsinger (1880) believed that in the horse, as in the pig, there were sympathetic sudomotor nerves contained in the infra-orbital branch of the fifth nerve, and that, as in the pig, sweating resulted when this was stimulated, but Bell & Evans (1956), in one experiment, could not confirm this. In any case, as the fifth nerve contains a large component of vasodilator fibres, any secretion resulting from stimulation could be explained on the same lines as that resulting from sympathetic section, as will be explained later. A further fact which supports this view is that after injury to, or section of, various nerves, or of the spinal cord, in horses, local sweating is often seen in the areas thus deprived of nervous connexion with the centres.

In the cat, there is an accumulation of cholinesterase on the surface of the cells of the eccrine sweat glands, and in association with the cholinergic nerve fibres by which they are innervated; in the horse the apocrine sweat glands contain amine oxidase, but this is distributed throughout the cells of the sweat glands, and is not associated with nerve fibres (Hellmann, 1955).

5. Humoral Control of Apocrine Glands

a. In the Horse

Since no sweat secretory nerve fibres seem to be demonstrable in the horse, we are left with the possibility of humoral control of sweating due to adrenaline present in the blood. This explanation appeared the more likely since it was found (Evans *et al.* 1956) that exercise such as to cause free sweating

was accompanied by an increase in the adrenaline content of the blood plasma—in horses at rest the plasma contains on the average, in England, 1.7 μg./l. of adrenaline; immediately after exercise the amount was increased by about 78%, and then declined quickly. The increase in concentration of adrenaline is considered to be adequate to cause sweating, since this can result from the intravenous injection of 0.5–1.0 μg./kg. Atropine (0.06 mg./kg.), although it lowers the resting level of adrenaline, does not prevent the rise on exercise, nor does it affect the sweating.

There is evidence also that Thoroughbred horses at rest in Malaya have a higher plasma adrenaline content than have resting hunters in England. This suggests that hot environments, as well as exercise, lead to a discharge of adrenaline into the blood stream, and that this may again be the actual stimulus to the sweat glands in hot environments (Evans, Ross, Smith & Weil-Malherbe, 1957).

If, as is claimed, the sweat glands in the horse have no sympathetic supply, either excitatory or inhibitory, how can the sweating which follows sympathetic section be explained? The simplest explanation seems to be that all sweating is largely dependent upon skin temperature, and upon the local blood supply, as has been shown by Burn (1922); increase in temperature, or in blood supply alone, is not competent to cause sweating, but apparently reduces the threshold stimulus which is required to cause it. In the eccrine glands this stimulus would be some parasympathomimetic agent, or a stimulus via the sympathetic sudomotor nerves. In the horse it would be adrenaline, which is already present in the blood. Hence, the local vasodilatation which follows sympathetic section, or stimulation of vasodilator nerves, by admitting more adrenaline, may overstep the threshold for it, and so cause spontaneous sweating. Also, since after sympathetic decentralization the area remains for months with a larger blood supply than other areas of the skin, so the increased blood adrenaline resulting from exercise affects that area sooner and more intensely than others.

An explanation of the sweating which results from intradermal injections of acetylcholine or similar agents may also be made along these lines. The local flushing of the skin is evident in pale-skinned horses, and precedes the local sweating; if atropine is given, neither occurs. Large intravenous doses of carbachol, or of acetylcholine preceded by an anticholinesterase, cause profuse general sweating, but this can be attributed to release of adrenaline from the adrenals.

b. In Man

The question of the relation of the sympathetic supply to the apocrine glands in man remains open. The researches of Shelley and his associates show that these glands are adrenergic, and that they respond to strong emotional stimuli such as would be expected to lead to release of adrenaline, but that they do not respond to a hot environment. Kuno (1956) considers that they also respond to mental stimuli. The indications are, therefore, that they resemble the apocrine glands of the horse in their responses, and the significant statement has been made (Shelley & Hurley, 1952) that sympathectomy does not impair their response to pain stimuli. What is wanted are further observations, under atropine, on the response to painful stimuli after sympathectomy and on the effects, at operation, of sympathetic stimulation. Such results are not forthcoming.

6. Refractory Phenomena in Sweating

It was noted by Langley & Bennett (1923) that, in the horse, an area of skin which had sweated in response to subcutaneous injection of adrenaline subsequently became refractory and gave no sweat response to a second injection, or to exercise, for some hours afterwards. A similar observation was made on human sweat glands by Sonnenschein *et al.* (1949).

The observation on horse sweating has been confirmed by Evans & Smith (1956), and was shown to occur also after sympathetic decentralization of the area. The phenomenon has been invoked to explain the tropical anhidrosis of Thoroughbred horses imported into Malaya (Evans, Ross, Smith & Weil-Malherbe, 1957). In these horses, the anhidrosis is preceded by profuse sweating, and comes on gradually. The condition, when partial, can be rendered complete for several days by administration of adrenaline intravenously. The " dry-coat " horses show little or no response to adrenaline intravenously or intradermally.

No fundamental explanation of this refractory state is at present forthcoming, but it is reversible after a few days, provided that the thermal stress is sufficiently reduced, as, for example, by lowering the temperature and especially the relative humidity of the surroundings (Ross, personal communication, 1956).

The reversibility of the condition of dry-coat in horses indicates that the condition is functional and not explicable as being due to atrophy of the sweat glands. Histological examination of the skins of free-sweating and dry-coat horses in Malaya fully supports this indication. Nearly all the glands in the skin of dry-coat horses are of normal structure, and about 10% of them are blocked by keratin plugs, most probably the consequence rather than the cause of the dry-coat state (Evans, Nisbet & Ross, 1957).

7. Summary

The classical conception of sweating as a cholinergic sympathetic phenomenon, as seen in the eccrine glands of man, cat and pig, needs qualification when the apocrine glands, as in the horse or in the human axilla, are considered.

There is no evidence that the apocrine glands are supplied with secretory nerves of any kind. They respond to adrenaline carried in the blood stream. The only influence which the sympathetic system exerts on them is an indirect one effected through changes in the local circulation.

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INNERVATION OF ABDOMINAL VISCERA

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1 Intrinsic nervous control of the gut
2 Afferent nervous impulses from the abdominal viscera
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References

The ancients believed that the function of the sympathetic division of the autonomic nervous system is to promote "sympathy", or co-ordination, among the visceral organs. This belief, if extended to both divisions of the autonomic nervous system and applied to the control of the vegetative systems of the body, is fully acceptable at the present day. Yet our understanding of the "machinery" of this control is far from complete: not only are there gaps in our knowledge but there are also downright inconsistencies in our experimental findings. Not infrequently belief has to be an act of faith.

1. Intrinsic Nervous Control of the Gut

> On no subject in physiology do we meet with so many discrepancies of fact and opinion as in that of the physiology of the intestinal movements.
>
> *Bayliss & Starling* (1899)

The gut exhibits co-ordinated and complicated movements independently of its extrinsic nerve supply. Cannon's (1912) description has never been bettered; there is segmentation, there are tone waves arising in tone rings, and there is peristalsis. In vitro the Magnus preparation of the gut of most animals shows rapid rhythmical activity superimposed on a slowly fluctuating basal tone. The Trendelenburg (1917) preparation exhibits peristalsis in vitro most beautifully. Somewhat casually all physiologists regard segmentation and tone waves as myogenic. Since the time of Bayliss & Starling's first description in 1899 we, in Britain, have regarded peristalsis as neurogenic, mediated by a reflex in the myenteric plexus of Auerbach: workers in the United States of America, however, think it quite unnecessary to invoke nervous activity to explain peristalsis (Alvarez, 1950).

However this may be, there are at least five nerve plexuses in the thickness of the gut wall (Garven, 1957). In 1927 Catherine Hill published a long paper on the histology of the nervous structures in the wall of the gut. From her knowledge of the abundant past literature on the subject and from her own observations she gave a description so persuasive and so consistent with the rather naïve beliefs of the time that it is still accepted by physiologists as the final and definitive word on the subject. Histologists know better: here is Meyling (1953)—

> . . . a clear and indisputable description of the organization of the nervous apparatus of the intestines, based on histological investigations, does not yet exist.

The claim of Magnus to have demonstrated rhythmicity and tone in portions of gut without nervous elements will no longer stand scrutiny. But that is not to say that rhythmical activity and tone of the gut are dependent on these nervous elements. With peristalsis it is otherwise. Evidence is accumulating to support the original idea that this activity is due to local reflex activity within the gut wall. Feldberg & Lin (1949) showed that the peristaltic reflex is abolished by cocaine and by D-tubocurarine. Paton & Zaimis (1951) confirmed this and showed that hexamethonium also prevented peristalsis. Kosterlitz, Pirie & Robinson (1956) find that, in the guinea-pig ileum, contraction of the circular coat, the major factor in peristalsis, is paralysed by ganglion-blocking agents but that the preparatory contraction of the longitudinal coat is not paralysed. Schaumann (1955, 1957) is convinced that there are nervous factors even in the preparatory phase of peristalsis, since he found that this phase is abolished by the presence of morphine. He claims that morphine acts by inhibiting the processes which release acetylcholine from the postganglionic neurones. Paton (1957), by stimulating guinea-pig gut with coaxial electrodes, comes to the same conclusion about the action of morphine. Yet we must in all honesty admit that we do not yet understand the way in which the nervous elements control peristalsis. As long ago as 1922 Langley postulated the existence of motor and of inhibitor cells in the myenteric plexus: Ambache (1951), by the use of botulinum toxin, which he believes to have a specific paralysing effect on cholinergic neurones, claims to show the existence of inhibitor adrenergic ganglion cells in the myenteric plexus. There the problem must be left until we have more knowledge of the intimate structure and functions of the nervous tissue within the gut wall.

Another aspect of the co-ordination of intestinal activity has recently been revived. In 1915 Keith brought forward histological evidence for the existence of pace-makers which control the activity of the gut. He found "nodal centres" of neuromuscular tissue at the gastro-oesophageal junction, in the proximal portion of the duodenum, at the ileo-colic junction and at certain points in the colon. Douglas (1949) now has evidence that the duodenum of dogs is indeed a rhythmogenic centre for the jejunum, and Milton & Smith (1956) find that the main "centre" for the duodenum and jejunum is near to the entry of the bile duct.

2. Afferent Nervous Impulses from the Abdominal Viscera

That stimulation of the viscera can give rise to sensation is all too obvious when mischief is afoot in the abdomen. Whether or not these sensations are "referred" is a somewhat sterile argument and has certainly not told us much about the nature and function of the impulses travelling in from the viscera. It is very probable that these impulses normally go quietly about their business without eliciting

sensation at all. Carmichael, Doupe, Harper & McSwiney (1939) caused vasomotor responses by distending the duodenum in man. Cunningham, Guttmann, Whitteridge & Wyndham (1953) elicited cardiovascular responses by distending the urinary bladder of paraplegic patients. They noted that the effective stimulus was pressure and not the volume of fluid within the bladder: they hazard the opinion that the function of this response is to ensure adequate blood flow in the wall of a distended viscus.

In 1925 McDowall showed that stretching of the gut, compression of the distended urinary bladder and scratching of the parietal peritoneum all caused dilatation of the pupil of the eye in cats under chloralose anaesthesia. McSwiney and his colleagues used this reaction to study the sensitivity of the various regions of the alimentary canal of cats: the gut was distended by a balloon. They also followed in some detail the routes taken by these impulses from the gut into the central nervous system (Irving, McSwiney & Suffolk, 1937; McSwiney & Suffolk, 1938). The in-going fibres lie both in the vagi and in the splanchnic nerves.

It has now become possible to record satisfactorily action potentials in afferent fibres from the viscera. Whitteridge (1956) has recently reviewed our information. Paintal (1954a, 1954b) recorded impulses from stretch receptors in the stomach of cats and studied the effect of certain drugs on these and other receptors in the viscera. Iggo (1955, 1956) also recorded impulses from single fibres in the cervical vagus of goats, of sheep and of cats. He was able to show that these impulses arose from tension receptors in series with the muscle fibres, since these receptors were excited by both passive stretching and active contraction of the stomach wall.

It is as yet difficult to say what role these in-going impulses play. As long ago as 1904 Macewen watched the behaviour of the caecum and colon through caecal fistulae in human beings. He noted that, immediately on taking food into the human stomach, the ileo-colic sphincter was inhibited and the activity of the distal ileum and of the colon was increased: hence the common experience of a call to defaecation shortly after a meal. The response, he says:

> . . . followed the ingestion of food too quickly to permit of the peristalsis acting through the whole length of the small intestine. It was, therefore, regarded as probably due to reflex action.

The existence of such a gastro-colic reflex of central origin has been questioned by Douglas & Mann (1939, 1940) and by Douglas (1948). They found that, in dogs, eating sent a wave of activity along the entire intestine, which reached the ileum in a very short time. This " post-prandial excitation of the intestine " was obtained after section of the extrinsic nerves to the gut and must therefore have been due to local intestinal factors. On the other hand, Gregory (1950) found, also in dogs, that, when food entered the stomach, the tone and motility of the jejunum was increased: this response was dependent on the extrinsic nerves to the gut. Such findings are in keeping with Macewen's original conception.

3. Nervous Control of Micturition and of Defaecation

The urinary bladder and the distal region of the colon have, to some extent at least, lost the autonomy so characteristic of most of the gut. There is central reflex control of which we now have a reasonably good understanding.

a. Micturition

Our present knowledge is to no small extent due to the painstaking dissections and observations by such pioneers as Griffiths (1891, 1895) and Langley & Anderson (1895b, 1896). Such work made possible the later more experimental approach of Elliott (1907) and of Langley himself (1911). But it was left to Barrington (1915, 1916, 1921, 1928, 1931) to elucidate in masterly fashion the functions of the nerve supply of the bladder and urethra in the cat. The impulses from bladder and urethra pass into the spinal cord along the pelvic nerves. These in-going impulses reflexly elicit outgoing impulses, again in the pelvic nerves: the outgoing impulses are motor to the detrusor muscle of the bladder and inhibitor to the internal sphincter of the urethra. At the same time there is cessation of the continuous train of impulses which, in the somatic pudendal nerves, maintain the steady tone of the striped muscle of the external urethral sphincter.

Barrington achieved his results without ever recording an action potential from a nerve. Evans (1936) was the first to record action potentials in filaments of the nerves passing to and from the bladder in the cat. His findings were completely consistent with the scheme put forward by Barrington. Iggo (1955) recorded afferent impulses in single fibres of the pelvic nerves of cats. The receptors were in the bladder wall and responded when the wall was stretched and also when the wall contracted.

b. Defaecation

The nervous control of defaecation resembles closely that of micturition. Garry (1933a, 1933b) obtained a nearly complete record of the component reflexes in cats. The centre is in the lumbo-sacral region of the cord. Recently a few further details have been added by taking electromyographic records from the external anal sphincter of cats (Bishop, Garry, Roberts & Todd, 1956). Distension of the colon throughout its length inhibited the tonic activity of the external anal sphincter. The in-going side of the reflex arc is in the pelvic nerves, the outgoing in the pudendal nerves. Section of the pelvic nerves destroyed the reflex and enhanced the resting tone of the external sphincter: this suggests the existence of a steady train in the pelvic nerves of in-going impulses from the viscera. One function may be to damp down the tonic activity of the external anal sphincter.

We have no picture at all of the tension receptors in the gut and in the bladder. Identification will depend on future collaboration between histologists and experimental physiologists.

There is little evidence that the sympathetic division of the autonomic system plays any vital role in micturition or in defaecation. Both acts are more easily evoked after division of the sympathetic supply to the pelvic viscera. Barrington describes one reflex confined to the sympathetic innervation of the cat's bladder. This reflex seems, however, to be unimportant for micturition; there is a weak and transient contraction of part of the detrusor muscle on distension of the proximal portion of the urethra.

4. Efferent Impulses in the Extrinsic Nerves to Abdominal Viscera

The results from stimulation of extrinsic efferent nerves to the viscera, both in vivo and in vitro, seem to show that two main factors influence the response. Low frequencies of

stimulation favour parasympathetic effects, higher frequencies sympathetic actions. The state of the effector also seems to affect the result. If the effector is active, if its tone is high, then stimulation of parasympathetic or of sympathetic outflows causes inhibition: if the initial state of activity is low, then the effect of stimulation, either of the sympathetic or of the parasympathetic outflow, is to increase the activity. There may be, then, a " peripheral mechanism " determining, independently of the origin of the extrinsic nervous impulses, the nature of the response.

a. To Stomach

McSwiney and his colleagues initially attributed a dominant role to the state of the " peripheral mechanism ". Indeed at one time they concluded that there is " . . . no evidence of any direct antagonism between vagus and splanchnic " (McCrea & McSwiney, 1928). Subsequently this school of workers began to place the emphasis on the effect of frequency of stimulation, the lower frequencies tending to give motor responses (McSwiney & Robson, 1929, 1931a, 1931b). There is also the suggestion that two types of muscle cell, with different responses, may exist in the stomach. Still later Harrison & McSwiney (1936) concluded that the anatomical vagi and the anatomical sympathetic nerves contain both adrenergic and cholinergic fibres. Recently Duncan (1953) found that total vagotomy in sheep destroyed the normal activity of the rumen and reticulum: rumination ceased and reflex closure of the oesophageal groove in lambs was abolished. Section of the splanchnic nerves had no significant effect on gastric motility.

b. To Small Intestine and Ileo-Colic Sphincter

Information about the effect of stimulation of efferent nerves to the small gut is sparse. The motor effect which Kuré, Ichiko & Ishikawa (1931) obtained from stimulation of the splanchnic nerves they attributed to the existence of spinal parasympathetic fibres. More significant is the work of Finkleman (1930). He stimulated in vitro the periarterial mesenteric nerves to the duodenum of the rabbit. Those nerves are " mixed " in the sense that they contain postganglionic sympathetic as well as preganglionic vagal parasympathetic fibres. Usually the effect was inhibition but, in fresh preparations and with a frequency of stimulation as low as 2–4 pulses/sec., he sometimes obtained a motor response. Alexander (1949) found that acetylcholine caused contraction of all regions of the gut of horses in vitro, and so did adrenaline in the majority of cases. When, however, the blood vessels of the gut were perfused with blood, then adrenaline decreased the tone of the ileum. Munro (1951, 1952, 1953) made a special study of the reactions of the small intestine of the guinea-pig for some few centimetres proximal to the ileo-colic sphincter. In vitro, adrenaline and acetylcholine both caused contraction; the motor action of acetylcholine was abolished by the presence of atropine, but not that of adrenaline. The presence of ergotoxine abolished the motor effect of adrenaline and revealed an inhibitor action. The effects of stimulation of periarterial mesenteric nerves were not so uniform: the motor response to such stimulation was sometimes abolished but sometimes enhanced by the presence of atropine: ergotoxine did not eliminate the motor response to nerve stimulation. It would be attractive to imagine that these findings in the distal ileum—they did not occur in more proximal regions of the small intestine of the guinea-pig—were due to the close proximity of the ileo-colic sphincter. Since the work of Elliott (1904) no one has questioned the motor effect of the sympathetic outflow on this sphincter: there is no satisfactory evidence, however, for a parasympathetic inhibitory action. One could imagine that, in the guinea-pig, the sphincteric mechanism extends proximally up the ileum for some distance, gradually giving way to muscle with reactions characteristic of the small intestine proper. Such a conception would be consistent with Munro's findings.

c. To Large Intestine

The distal region of the colon is innervated by the pelvic nerves which contain the sacral parasympathetic outflow. The sympathetic supply is from the lumbar splanchnic outflow. There is said to be considerable intermingling of sympathetic and parasympathetic fibres in the nerve strands close to the colon.

Langley & Anderson (1895a) found that stimulation of the pelvic nerves of the rabbit in vivo gave contraction of the colon, but stimulation of anatomically sympathetic strands close to the bowel could produce motor and biphasic responses as well as the more common inhibition. Such variable responses they attributed to the presence of parasympathetic fibres of sacral origin in the nerve strands.

Garry (1933a), working on decerebrate cats, found that stimulation of the pelvic nerves invariably gave contraction of the colon and inhibition of the internal anal sphincter of smooth muscle. Stimulation of all parts of the sympathetic outflow caused, with equal certainty, inhibition of the colon and contraction of the anal sphincter. Moreover, again in the cat, spinal anaesthesia or division of the sympathetic supply led to intense activity of the colon and relaxation of the internal anal sphincter. In the guinea-pig, however, Munro (1953) found that stimulation of periarterial mesenteric nerves could give either motor or inhibitor responses: the presence of tone favoured an inhibitor response.

Garry & Gillespie (1955) worked with an innervated in-vitro preparation of the distal colon of the rabbit. With this preparation it is possible to stimulate, by fluid electrodes, either the sympathetic strands to the colon or the pelvic parasympathetic fibres, or both outflows together. They hit upon the fact that the sensitivity of the two outflows to stimulation varied. Moreover, the characteristics of the two responses were quite different. The response to stimulation of the pelvic nerve was a rapid contraction not well sustained. Low frequencies of stimulation, of the order of 10 pulses/sec., were the most effective. It was shown conclusively that the relative ineffectiveness of the higher frequencies was not due to fatigue. The inhibition in response to stimulation of the sympathetic outflow was slow in onset, well sustained, and persisted for a considerable time after cessation of the stimulation. Low frequencies of stimulation, 10 pulses/sec. or less, were relatively ineffective in causing inhibition: the most effective frequencies ranged from 50 to 100 pulses/sec. Whatever the initial state of tone of the preparation, stimulation of the parasympathetic outflow always gave contraction, and stimulation of the sympathetic outflow always caused relaxation.

The presence of hexamethonium or of atropine blocked the response to stimulation of the pelvic nerves without affecting the response to stimulation of the lumbar outflow.

Finally, the sympathetic and parasympathetic outflows were stimulated simultaneously. At low frequencies, say at 2 pulses/sec., the response was purely motor; at intermediate frequencies, 10–50 pulses/sec., the response was biphasic, first a contraction and then a relaxation; but, with frequencies greater than 100 pulses/sec., there was only relaxation. Thus stimulation of a nerve, known to have a mixture of sympathetic and parasympathetic fibres, gave responses similar to those obtained by many past workers when stimulating nervous strands to other parts of the gut.

d. To Urinary Bladder

The bladder and the internal urethral sphincter of smooth muscle have an innervation not unlike that of the distal portion of the large gut. Once more we owe most of our information to the meticulously careful dissections and observations of Langley & Anderson (1895b). Elliott (1907) followed with a no less careful study and emphasized what may be a most important feature not only in the bladder but elsewhere in the abdominal viscera. Stimulation of the peripheral ends of cut pelvic nerves had a motor action on the bladder wall and inhibited the internal urethral sphincter. However, while stimulation of the peripheral ends of the hypogastric nerves, part of the sympathetic outflow, was motor to the internal urethral sphincter, the effect on the bladder wall was biphasic: there was an initial motor action followed by inhibition. Inspection showed that this motor effect was due to a contraction localized to smooth muscle, spreading out over the trigone of the bladder from the internal urethral sphincter. Ergotoxine abolished the motor action but left unimpaired the inhibitory effect. In the cat, dog and rabbit this sheet of muscle with a motor supply from the hypogastric nerves is not extensive, but in the ferret it seems to spread over the entire bladder. In that animal, stimulation both of the hypogastric nerves and of the pelvic nerves gave a powerful contraction of apparently the entire bladder wall. Here is a dramatic example of species difference. Barrington's third micturition reflex in the cat, where distension of the urethra evokes a slight transient rise in intravesical pressure, must be due to the reaction of this special sheet of muscle innervated by the hypogastric nerves.

In 1933 Gruber wrote an extensive review of the autonomic innervation of the genito-urinary system. There, in Table I, can be seen at a glance the variable responses of the bladder when the hypogastric and the pelvic nerves were stimulated by many workers. However, more recent findings conform to the conception that the parasympathetic outflow is cholinergic and motor to much of the detrusor muscle, while the sympathetic outflow is adrenergic and inhibitor to this same muscle. Edge (1955) brings the story up to date and shows that the hypogastric nerves are adrenergic.

e. To Uterus

In spite of the careful dissections of earlier workers, there is still doubt about the morphology of the nerve supply to the uterus. Reynolds (1949) and Schofield (1952) suggest that the nerves control primarily the blood vessels and glands. In the great majority of cases the sympathetic outflow is motor in action, and the chief route is by the hypogastric nerves. The results from stimulation of the pelvic nerves are most variable. Gruber (1933) gives a comprehensive review of the earlier work. Recently it has been found that stimulation of the hypogastric nerves consistently causes contraction of the uterine cornua and of the cervix of rabbits (Schofield, 1952; Varagić, 1956). Mann & West (1951) stimulated the hypogastric nerves and recovered adrenaline and noradrenaline from the blood leaving the uterus of cats. Schofield (1952) obtained a motor effect in four out of 20 rabbits, by stimulation of the pelvic nerves.

The puzzling fact has long been known that pregnancy in the cat reverses the response of the uterus to stimulation of the hypogastric nerves and to adrenaline and noradrenaline. In this instance, at least, it is justifiable to place responsibility for the change squarely on the peripheral mechanism.

5. General Comment

The conception of a nice balance between the sympathetic and parasympathetic divisions of the autonomic nervous system is part of our current thought in physiology. We are loath, and probably rightly so, to abandon this conception although so many results from experimental stimulation of nerves give it the lie.

One fundamental difficulty is the crudity of the experimental approach. It is surely naïve to open the abdomen, to cut a visceral nerve, to blast the peripheral end of the nerve with an electrical current of unknown nature and then to expect a result indicative of the normal function of that nerve. We shall remain in doubt until we can record from individual nerve fibres the impulses normally passing to and from the viscera. In the meantime, results from nerve section, due to loss of normal impulses, are likely to be more rewarding and revealing.

It is not often realized how defective is our knowledge at the cellular effector level. We lack precise information about the nature of smooth muscle cells. Are they all alike? We do not know how the nerve fibres of the autonomic system terminate in the viscera. The postulate of Boeke (1940) that there is an interstitial cell net between the autonomic nerves and the effectors is gaining ground: here is his " Umwertungsstelle der Erregung ". This " peripheral mechanism " may well play a part in determining the response, and it may be vulnerable to extraneous influences in ways which we do not so far realize.

Finally, our knowledge of the composition of the strands of nerve fibres supplying the viscera is far from satisfactory. Are the outgoing fibres in the anatomical vagus all parasympathetic? Are the outgoing fibres in the splanchnic nerves all sympathetic? Recent anatomical work by Mitchell (1953) suggests that many visceral nerves are " mixed " in the sense that they contain sympathetic and parasympathetic fibres. Garry & Gillespie (1955) believe that their experimental findings on the colon support this idea and help to explain away the disconcerting capriciousness of many of the results of past workers when stimulating nerves to other parts of the gut. It is probably significant that confusion usually is most obvious where the approach is least physiological. It would be sheer defeatism to admit at this stage a principle of uncertainty in the innervation of the abdominal viscera.

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INTERMEDIATE SYMPATHETIC GANGLIA

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1 Sudomotor "escape areas" following sympathectomy
2 The intermediate ganglia
3 Implications of the presence of intermediate ganglia
References

Surgical extirpation of portions of the sympathetic nervous system frequently fails to produce precisely that permanent peripheral denervation which is to be expected from text-book diagrams of the anatomical arrangement of the system. In particular the regional extent of the diminution in sympathetic function is often distinctly less than what might be expected. The discrepancies are not uncommon even when the surgical technique is above suspicion. They can in part, of course, be attributed to variations in the detailed anatomy of the sympathetic trunks or in the pattern and distribution of the branches and communications of these trunks. Such atypical arrangements in the autonomic nervous system are frequent. Thus, for example, during careful dissection of the cadaver, with all the relationships exposed, the correct identification of a particular paravertebral sympathetic ganglion can be very difficult; in the depths of a surgical incision, it is often impossible. But, even when the possibility of the usual anatomical anomalies of the sympathetic nervous system has been excluded, persistence of autonomic activity in unexpected areas may, and in certain regions always does, follow operative removal of parts of the system which should have caused complete sympathetic paralysis in the area or region concerned. It has been shown that these results can readily be explained by the regular presence of peripheral sympathetic ganglion cells in positions where they cannot themselves be removed by the operative procedure adopted. Often, too, the anatomical relationships are such that the fibres (postganglionic) from these cells are likely to escape, in whole or in part, from the traumatization effects of the classical operations for sympathectomy. It is with the distribution and relationships of such cells, which, when in groups, have come to be called, rather unfortunately, intermediate ganglia, that this communication is concerned.

Furthermore, in introduction, it can be pointed out that, after due allowance has been made for the rapidity of regeneration of cut sympathetic nerve fibres, there are statements in the literature on the restitution of autonomic function following sympathectomies which are, at least, most puzzling. Indeed, in some instances, they suggest the miraculous. The sympathetic ganglion cells to which reference has just been made, and which, as will be shown, are unlikely to be removed by the surgeon, may also have an importance in the explanation of some of these unexpected restitutions of function and of surprising "regenerations" following sympathectomies.

1. Sudomotor "Escape Areas" Following Sympathectomy

More than 25 years ago Brown & Adson (1930) reported on the physiological effects of thoracic and lumbar sympathetic ganglionectomies in a number of human subjects. They include accounts of the effects of the operations on the pattern of sweating which followed the heating of patients in a chamber. Among their observations were some on patients who had been subjected to bilateral removal of the second, third and fourth paravertebral sympathetic ganglia. These investigators recorded, though they did not comment on, persistence of sweating in the second and third lumbar dermatomes, notwithstanding the removal of the sympathetic ganglia of these segments. In the 15 years following the report by Brown and Adson, little was added to their data. While the special difficulties of achieving complete sympathetic denervation of the upper extremity of man were generally realized, such denervation of the lower extremity was, apparently, believed to offer no special problem. Thus Smithwick (1940) wrote: "... removal of the first, second and third lumbar ganglia results in complete sympathetic denervation of the thigh and leg." In 1947, however, Richter reported on the cutaneous distribution of sympathetic nerves, as determined by the "skin resistance method", in a large series of patients who had undergone extensive thoracicolumbar sympathectomies. The findings in these patients showed that the operations had resulted in denervation of the sweat glands in a wide band round the trunk and in large areas on the legs. These denervated regions, however, were separated by an area where denervation had not occurred ("escape area"). The denervated areas on the legs had the same patterns as those previously found in patients subjected to lumbar sympathectomy. Richter further recorded that denervated trunk areas following thoracicolumbar sympathectomies had one feature in common: the location of their lower borders in the T12 dermatome. This pattern he found to be constant regardless of how many lumbar ganglia were removed.

Richter, in this contribution, stated that he had no satisfactory explanation for the existence of the non-denervated escape areas round the middle of the body. He listed several alternatives: (i) "Connections may exist in this part of the body ... about which at present we have no knowledge." As his data suggested that operations which extended far enough caudally to include the third and fourth lumbar ganglia leave smaller non-denervated escape areas immediately following operation, Richter expressed the opinion that if such "unknown connections" existed they must have their origin in the lower lumbar ganglia. (ii) The escape areas may show compensatory sweat gland activity, owing, he suggested, to the associated denervation of large areas of skin on other parts of the body. (iii) The remaining lumbar ganglia isolated from preganglionic connexions may assume a local control.

In the reported discussion following the paper of Richter to which reference has just been made, Ray drew attention to points relating to the residual sympathetic innervation which are discussed in full in his paper with Console (1948). In this contribution Ray and Console recorded observations which led them to the "inevitable conclusion" that the twelfth thoracic and first three lumbar dermatomes receive part of their sympathetic nerve supply "through pathways that do not pass through any part of the paravertebral chain from

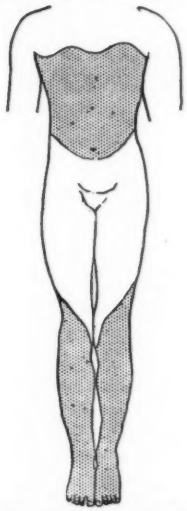
Th.8-L3." These investigators further indicated that the apparently complete sympathetic denervation of the lower extremity is a temporary phenomenon. It is present only until readjustment takes place and the functional activity of the residual pathways becomes manifest. Their results showed quite clearly that complete denervation of the lower extremity is not determined by preservation or removal of the first lumbar ganglion nor of any portion of the paravertebral sympathetic chain above this level.

Paravertebral sympathectomy from the level of the stellate ganglion to—and including—the third lumbar ganglion was found to have no effect on the sweating pattern in the lower extremity. Consequently Ray and Console concluded that residual pathways by way of the nerves in the vertebral canal could be eliminated in any attempt to explain their findings. On the other hand they concluded that the residual pathways to the escape area must be connected with the anterior spinal roots of the segments (T12-L3) involved. As they were able to show that the residual pathways could be blocked by tetraethyl ammonium chloride, preganglionic and postganglionic fibres must be involved. On the position of the ganglion cells concerned in the pathway, Ray and Console expressed themselves as uncertain; it was suggested, however, that the cells might be " . . . in the plexus about the aorta, behind the psoas muscle, or in the lumbar nerves themselves ". In any case, in their opinion, the postganglionic fibres are not distributed to the related dermatomes by way of the periarterial sympathetic nerves. These fibres of the residual pathway must join the nerves of the lumbar plexus near their origins and pass to the dermatomes included in these nerves. Finally, in this important paper, Ray and Console suggested that there may be similar (residual) pathways distributed to the face, the lower sacral dermatomes, the axilla and T1-T2 dermatomes and scattered areas on the trunk.

Shortly after the appearance of the Ray and Console communication, A. M. Boyd (1948) reported observations on patients after sympathectomy which were comparable with those of the American workers. In spite of the cogency of the arguments in favour of residual pathways advanced by Ray and Console, however, Hyndman, Wolkin & van Allen (1948) criticized these two investigators for postulating a system of fibres hitherto unrecognized in the literature.

Meanwhile Dr P. A. G. Monro, who was then a member of the Anatomy Department at the London Hospital Medical College, had been investigating the sudomotor pattern, following induced sweating, in patients who had had paravertebral sympathectomies, and who were under the care of Mr D. W. C. Northfield in the neurosurgical unit at the London Hospital. Dr Monro was readily able to verify the regular occurrence of a sudomotor escape belt in the dermatome segments of T12-L3 after such operations at relevant levels (fig. 1). These findings suggested to Monro and to me, as they had to Ray and Console, that an alternative sudomotor pathway to the first three lumbar dermatomes persists intact after the operation. This led us (Boyd & Monro, 1949) to examine serially sectioned human embryos and foetuses in an attempt to locate the sympathetic cells of the residual pathway, which are not removed in thoracicolumbar sympathectomy. This investigation revealed the presence of numerous—and constantly present (though variable in number and situation)—autonomic cells and ganglia in the course of the rami communicantes joining the anterior primary rami of the lumbar nerves to the corresponding anterior primary rami of the

FIG. 1. DIAGRAMMATIC REPRESENTATION OF THE LOSS OF REFLEX SUDOMOTOR ACTIVITY AFTER THORACICOLUMBAR SYMPATHECTOMY (T4-L3)

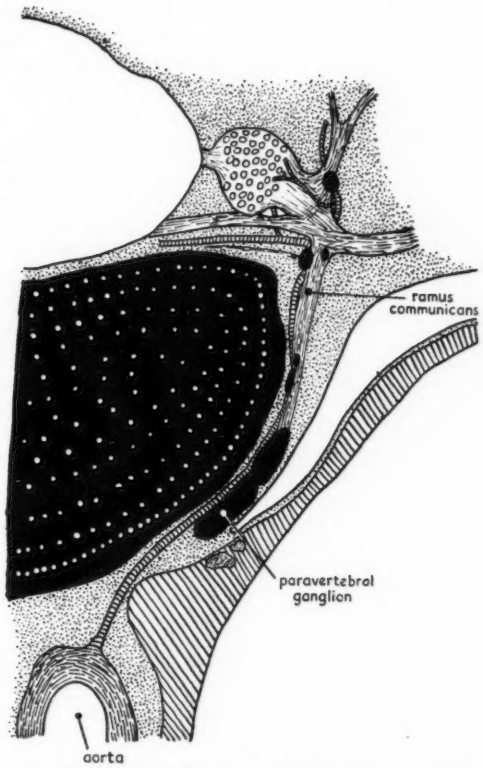


Absence of sweating on heating is indicated by stipple.

lumbar paravertebral sympathetic ganglia (fig. 2, and Plate I). We found the collections of autonomic cells which escape the surgeon's knife in lumbar paravertebral sympathectomy to be situated for the most part dorsal to the psoas muscle or between its posterior fibres of origin. We also found them to be situated along the course of the more dorsal of the rami communicantes of the first three lumbar nerves. These dorsal rami are those which are generally accepted to be of the grey variety. The collections of autonomic cells were found, variably, at any point along a ramus from its sympathetic ganglionic commencement to its junction with the spinal nerve. Some of them, indeed, could be found on the surface of—or embedded in—the anterior primary rami of the lumbar nerves themselves. Similar ganglia were also found on the rami communicantes—which are normally, of course, in man only of the grey variety—of the fourth and fifth lumbar nerves.

The site of these ganglia was such that it was apparent that they would inevitably escape being removed in the usual operation of thoracicolumbar paravertebral sympathectomy. We suggested, therefore, that such ganglia, with their associated preganglionic connexions and postganglionic fibres, provide the anatomical explanation for the retention of sudomotor activity in the escape belt shown by the first three lumbar segmental dermatomes after such operations. Moreover we were able to add a brief note on post-mortem material from one patient in the London Hospital series who had had paravertebral sympathectomies including each third lumbar ganglion. After the operations Dr Monro had been able to demonstrate a sudomotor escape pattern in this patient essentially similar to that described above. Histological examination of the material from this patient amply confirmed the presence of many intermediate ganglia in the rami communicantes and the anterior primary rami of the first and second lumbar nerves. In these persisting ganglia most of the autonomic cells appeared histologically normal, though some

FIG. 2. CAMERA LUCIDA DRAWING OF SECTION THROUGH THE SECOND LUMBAR VERTEBRA IN A 100 mm. CROWN-RUMP LENGTH HUMAN FOETUS



Six intermediate ganglia are shown in relation to the lumbar nerve and to a ramus communicans. The largest of the intermediate ganglia is almost confluent with the paravertebral sympathetic ganglion. Note intermediate ganglion related to the posterior primary ramus.

showed alterations which we thought were suggestive of degeneration. We suggested that this degeneration may have been retrograde, due to section of the corresponding postganglionic fibres. Subsequently Monro (1950, 1951a, 1951b) furnished further data on the material from this case. His more detailed examination revealed the presence of intermediate ganglia associated with the nerve roots of the first four lumbar nerves of each side of the body. Careful dissection of these nerves and of their rami communicantes on the left side was followed by block impregnation with a reduced silver technique so that an attempt could be made to trace the connexions of the persisting autonomic fibres. Monro established that the ganglia were associated with both the grey and the white rami communicantes of the first and second lumbar nerves. He found that many of the ganglion cells on the white rami showed degenerative changes, which he presumed was due to their postganglionic fibres' having been sectioned during the operation. Most of the cells in the ganglia at the base of the grey rami, however, appeared to be histologically normal. Some of their branches could be traced along the lumbar arteries towards the aorta, and the fibres in such branches had, presumably, escaped section. From his investigation Monro concluded that a thoracicolumbar paravertebral sympathectomy may not be sufficient completely to remove the relevant abdominal viscera and blood vessels from the influence of the sympathetic nervous system. Further, such an operation almost invariably fails to produce sympathetic denervation, as assessed by sudomotor activity, in the upper lumbar dermatomes.

2. The Intermediate Ganglia

The investigations referred to in the previous section demonstrate quite clearly that residual autonomic activity, following sympathectomy, in at least one part of the body can readily be explained by the presence of widely scattered sympathetic ganglia which are not removed by the classical sympathetic ganglionectomy operations. What is remarkable is that a significant literature on such scattered ganglia was, in fact, available to explain the atypical loss of autonomic function following such operations. This literature, however, was obviously unknown to Richter and to Ray and Console. Indeed Monro and I became aware of it only after we had ourselves located the position of the nerve cells to which we attributed the origin of the residual sympathetic pathways indicated by the clinical findings.

As early as 1845 Cruveilhier had described the presence of small enlargements on the course of the rami communicantes in man. He wrote: " Une disposition fort remarquable des rameaux de communication entre les paires et les ganglions lombaires, c'est la presence de ganglions ou renflements sur le trajet de ces rameaux . . ." Kölliker (1850) also recorded the occasional presence of ganglion cells in the course of rami communicantes. Later in the nineteenth century Onodi (1886) described such ganglia in the rami communicantes of human embryos. In the present century numerous further observations on such ganglia in man and other vertebrates were made by Marinesco & Minea (1908); van der Broek (1908); Hirt (1921); Romankevic (1930); Villemin & Dufour (1930); Rossi (1931); Gruss (1932); and Fagarasanu (1938). It was Hirt who introduced the term " intermediate ganglia " to describe the collections of autonomic nerve cells he found on the rami communicantes in certain reptiles. It is, however, above all to Wrete (1935, 1941, 1943a, 1943b, 1951) and to Skoog (1947) that we are indebted for most of our knowledge on the distribution of the intermediate ganglia. Kuntz & Alexander (1950); Boyd (1950, and unpublished observations); Ehrlich & Alexander (1951) and Monro (1951a, 1951b, 1954b) have all added detail to the accounts given by Wrete. Implicit in the last-named investigator's data, however, is the anatomical explanation of the residual sympathetic pathways. Indeed in his contribution on the intermediate ganglia in laboratory mammals Wrete (1941) indicates explicitly the possibly significant consequences of the presence of such ganglia on the interpretation of the results of sympathectomies in these animals. And in his 1943a paper, on the intermediate ganglia of the lumbar region in man, he suggests that a similar viewpoint should be taken in regard to clinical sympathetic ganglionectomy.

As to the distribution of the intermediate ganglia themselves, the findings of all the above-named investigators indicate that they can be divided into two principal groups. There is a cranial group of ganglia lying in the cervical and

uppermost thoracic segments and a caudal group related to the lumbar and lowermost thoracic segments. In the mid-thoracic and sacral regions, the intermediate ganglia are rare or, often, completely absent. For the most part they are on the course of the rami communicantes and most commonly on the postganglionic or grey rami. This last fact can be associated, perhaps, with their relative absence in the mid-thoracic region where the grey rami are very fine. The preganglionic connexions of the cells of the intermediate ganglia have yet to be established in detail. Certainly the results of section of the lumbar sympathetic chain below the level of the preganglionic outflow can be interpreted as indicating that the preganglionic fibres to the intermediate ganglia associated with the fourth and fifth lumbar nerves pass caudally in the paravertebral sympathetic chain itself. For the cervical intermediate ganglia the evidence is not so conclusive, but again it appears likely that most of their preganglionic fibres pass up in the cervical chain.

In addition to the intermediate ganglia associated with rami communicantes, similar ganglia are found in or near the anterior roots of the spinal nerves in the cervical and the lumbar region. In the sectioned embryos and foetuses available to me I have, at one time or another, found such autonomic ganglia in the actual substance of all of the cervical and all of the lumbar nerves. In the lumbar region such collections of cells are nearly always in the anterior primary ramus near the point of attachment of the rami communicantes, but they may be in a more dorsal situation close to the dorsal root ganglion. On two occasions I have found such ganglia on the posterior primary ramus of a lumbar nerve. In the cervical region, as the results of Skoog's investigations (Skoog, 1947) and my personal observations show, the intermediate ganglia tend to be more widely dispersed. In several embryos, indeed, such ganglionic aggregations have been found in the lower cervical nerves as they pass between the scalene muscles on their way to the brachial plexus. In no embryo or foetus, however, have autonomic cells been found in any of the spinal nerves contributing to this plexus distal to the level of the first rib.

It is, of course, hazardous to comment on the course of the preganglionic fibres to the intermediate ganglia, whatever may be their situation. For those cells situated in spinal nerves containing a preganglionic outflow it is, perhaps, justifiable to conclude that the preganglionic connexion is restricted to that nerve and that the postganglionic fibre passes distally in the nerve and in one of its branches until it leaves as a vascular (or sudomotor or pilomotor) fibre in the periphery. For the intermediate ganglia situated on the rami communicantes at levels of preganglionic outflow there are several possibilities which are summarized in fig. 3. Doubtless more than one, and possibly all, of these possibilities do occur as effective communications in life. For the intermediate ganglia in the lumbar region below the level of the preganglionic outflow most and probably all of the preganglionic supply passes caudally in the paravertebral chain. By analogy it might be expected that all the preganglionic supply to the cervical intermediate ganglia reaches the cells of these ganglia by way of the cervical paravertebral chain. The complications in this region however, introduced by the presence of the vertebral nerve, or nerves, by the presence of the ansa subclavia and by the frequent atypical arrangements of the sympathetic chain in front of the first rib, make an analysis here much more difficult. What seems quite clear is that in the lumbar region many of the preganglionic fibres to the intermediate ganglia escape section in thoracicolumbar sympathectomies. In the cervical region at least some of these fibres may escape in cervico-thoracic sympathectomies.

Lastly, in this brief description of the autonomic ganglia which may be left behind after sympathectomy, attention should perhaps be directed to sympathetic autonomic cells in the head and neck region, which will not be removed by cervical sympathetic ganglionectomy and which are not concerned with the postganglionic supply to the arm. These ganglia include those related to the more cranial cervical nerves and which are strictly comparable with the intermediate ganglia already described. There is a particularly large ganglion of this nature situated on the ramus communicans which joins the superior cervical ganglion to the second cervical nerve. Fibres from it can readily be traced in serial section into the great occipital nerve and also from this ganglion, or from an equivalent one on the third cervical ramus communicans, into the great auricular and lesser occipital nerves. The regular presence of these intermediate ganglia makes it clear that a superior cervical ganglionectomy in man will not result in complete removal of postganglionic sympathetic fibres to the back of the scalp or the ear. Examination of more limited material suggests that a similar situation obtains in the rabbit.

Aggregations of sympathetic cells of a presumably equivalent nature, though they are not associated with spinal rami communicantes, are found along the course of the internal carotid artery, in the walls of the cavernous sinus and in the orbit. They are also found along the external carotid artery and its branches. Thus I have found them on the superior thyroid artery, on the internal maxillary artery and on the middle meningeal artery. All the cells included in this group will, with their postganglionic fibres, survive even the most radical superior cervical ganglionectomy, though they will, of course, be cut off by such an operation from their preganglionic supply.

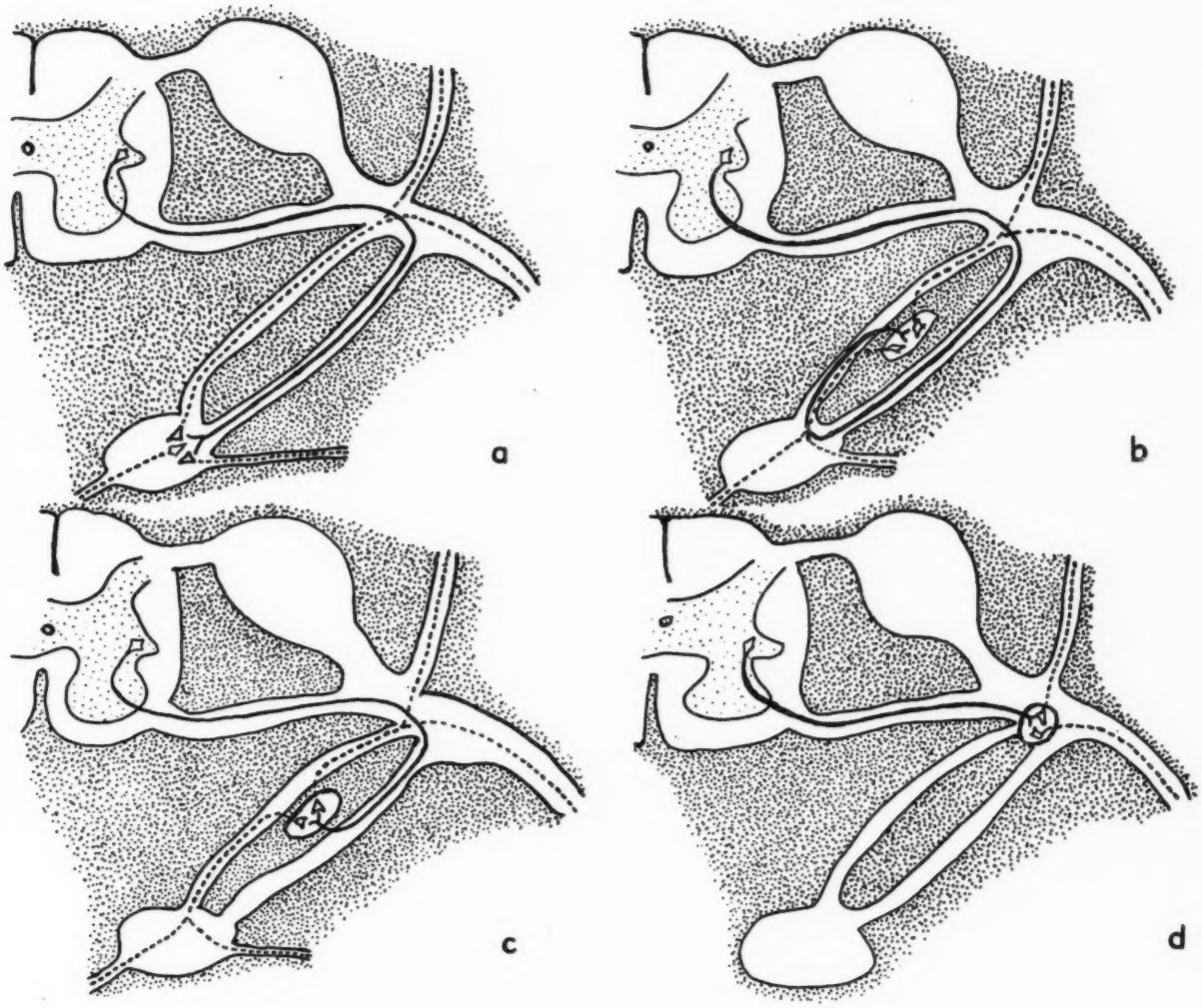
3. Implications of the Presence of Intermediate Ganglia

Apart from the obvious significance of the intermediate ganglia in the explanation of residual pathways after sympathectomies, and their importance in demonstrating the status of anatomical detail, these scattered aggregations of autonomic nervous cells would seem to have other implications.

In the first place the persistence of non-myelinated nerve fibres in a nerve trunk following a relevant paravertebral sympathectomy does not allow of the conclusion that those fibres are necessarily of dorsal root ganglion. This fact renders suspect all the work, such as that of Ranson & Davenport (1931), which has been widely influential, in which it has been assumed that all the autonomic postganglionic fibres are readily removed from a limb nerve by ablation of the sympathetic chain. These investigators based their conclusions largely on studies of the saphenous nerve and the nerve to the vastus medialis which are, of all those in the body included in the group, most likely to possess postganglionic fibres after removal of the lumbar sympathetic ganglia.

Secondly, although many (or even all) of the preganglionic fibres to many of the intermediate ganglia will be interrupted by relevant classical sympathectomies, the postganglionic fibres will often remain intact. Hence the histological identification

FIG. 3. POSSIBLE COURSES OF PREGANGLIONIC (continuous lines) AND POSTGANGLIONIC (interrupted lines) FIBRES IN RELATION TO CELLS OF INTERMEDIATE GANGLIA



a: connexions solely to paravertebral ganglion; the preganglionic fibre is in the white ramus (more ventral) and the postganglionic fibre is in the grey ramus

b: an intermediate ganglion receives a preganglionic fibre by way of the white ramus and the paravertebral ganglion. Such a fibre will be interrupted by a paravertebral sympathectomy

c: intermediate ganglion receives a preganglionic fibre directly from the white ramus. Such a fibre is likely to escape in a paravertebral sympathectomy

d: intermediate ganglion situated in spinal nerve. The preganglionic fibre in this instance can be interrupted only by section of the nerve or by anterior rhizotomy.

of persisting nerve fibres or endings in a tissue or organ does not permit, without careful anatomical analysis, of the conclusion that such persisting nervous elements cannot be sympathetic in nature. The importance of this statement in the study of amine oxidase activity will be obvious.

Finally the persistence of intact sympathetic cells with their postganglionic fibres doubtless explains many of the functional regenerative phenomena following sympathectomies. The anatomical situation may be such that sectioned preganglionic fibres can readily extend into the region containing the intermediate ganglia. Further, Geohegan & Aidar (1942), Monro (1954a, 1954b) and Murray & Thompson (1956, 1957)[1] have suggested that functional reorganization, possibly by the development of collateral sprouts, can occur after sympathectomy. This raises the possibility, as has been indicated by Monro (1954b) and Murray & Thompson (1957), that persisting intermediate ganglia may be of importance in providing the postganglionic pathway if functional contact with their cells is made by such collateral sprouts.

[1] See also Murray & Thompson, p. 213 of this number of the Bulletin.—Ed.

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* * *

PLATE I (FIGS. A—D)

PHOTOMICROGRAPHS OF SECTIONS THROUGH HUMAN FOETUSES TO SHOW LUMBAR INTERMEDIATE GANGLIA

(FIGS. A—D)

J. D. Boyd

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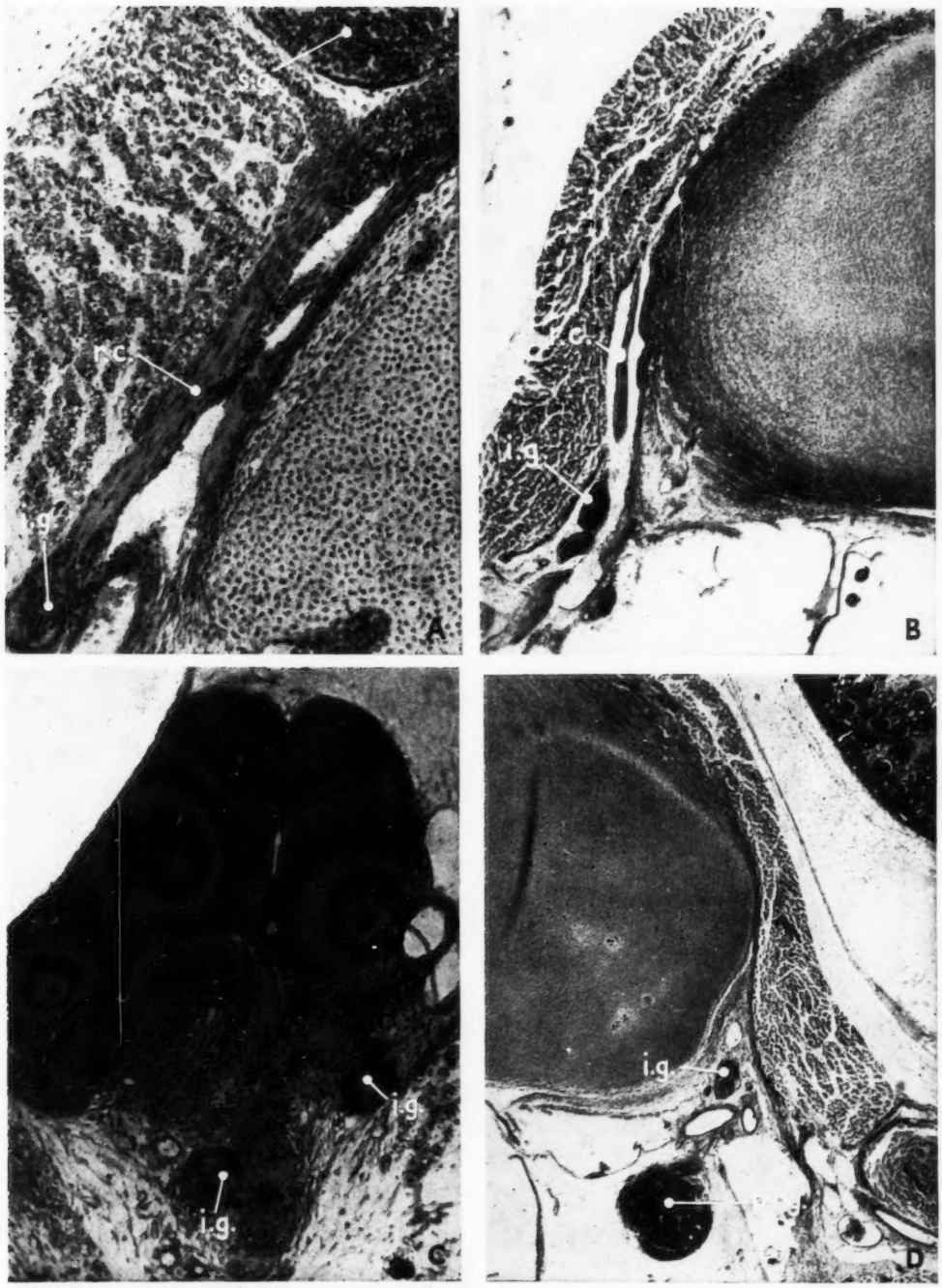

FIG. A. Intermediate ganglion in a ramus communicans
FIG. B. Intermediate ganglion close to an anterior primary ramus
FIG. C. An intermediate ganglion embedded in the anterior primary ramus of a lumbar nerve. Another small autonomic ganglion is lying on the surface of the nerve trunk
FIG. D. Large intermediate ganglion situated posterior to the psoas muscle at the dorso-lateral angle of the body of a lumbar vertebra

i.g.: intermediate ganglion
r.c.: ramus communicans
p.r.g.: posterior root ganglion
s.g.: sympathetic ganglion

PLATE II (FIGS. A—D)

LONGITUDINAL SECTIONS OF SUPERIOR CERVICAL GANGLIA OR PREGANGLIONIC TRUNKS, STAINED BY THE BIELSCHOWSKY METHOD

(FIGS. A—D)

Murray & Thompson

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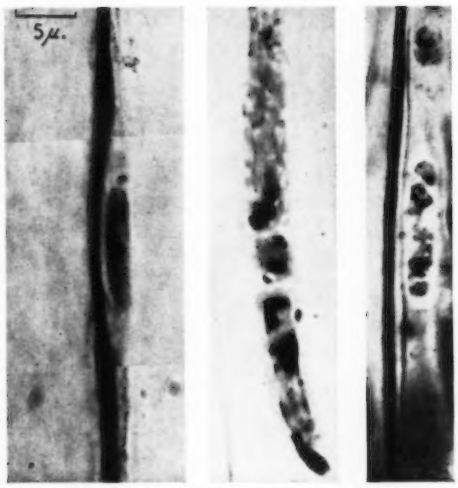
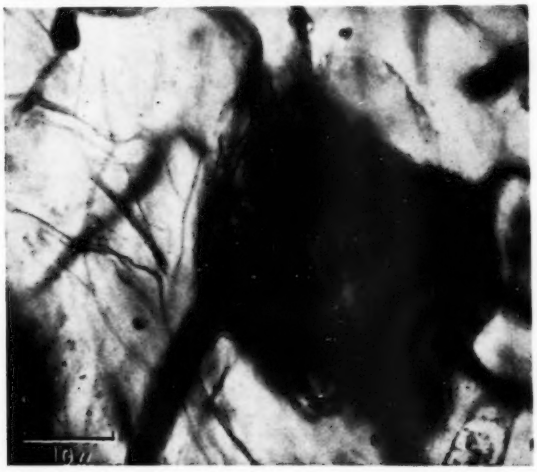


FIG. A. Sections of the preganglionic trunk 1 cm. caudal to the superior cervical ganglion 5 days after division of rami T1–3
a: a single intact preganglionic fibre with a Schwann cell nucleus and faintly stained, the Schwann cell cytoplasm around the axon
b: a Schwann tube filled with the debris of a degenerating axon
c: a single intact preganglionic fibre with an adjacent Schwann tube filled with debris; between the two is a fine collateral sprout which has arisen from the intact axon

FIG. C. A ganglion cell adjacent to the area shown in fig. B with nucleus, nucleolus and dendrites. At top left is a fragment of a preganglionic termination with a rounded mass of axoplasm in the process of degeneration. Several ring-like appearances, typical of degenerating fine preganglionic terminations, can be seen

FIG. B. Section within a ganglion 5 days after division of rami T1–3. A sprout has arisen more proximally from a parent intact preganglionic axon, grown into an adjacent Schwann tube still containing debris (top left) and been guided down the tube. A Schwann cell nucleus in the tube can be recognized. The sprout in turn divides into further sprouts and the point of division of one can be seen. On one sprout (bottom right) a bulbous appearance typical of a very early regenerating fibre can be seen. At bottom right is a Schwann tube filled with debris and a tiny new sprout running alongside

FIG. D. Same cell as in fig. C at a slightly different focus showing tiny sprouts, some coming into close apposition with the cell surface and dendrites



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COLLATERAL SPROUTING IN RESPONSE TO INJURY OF THE AUTONOMIC NERVOUS SYSTEM, AND ITS CONSEQUENCES

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1 Sprouting in the sympathetic nervous system
a Histology
b Functional efficiency of the partially denervated ganglion after sprouting
2 Time-course of the sprouting process
3 The stimulus to sprouting
4 Factors controlling the effectiveness of sprouting
5 Changes in pattern of autonomic innervation
6 Some applications
References

Until recently, it was generally supposed that, if a nerve is interrupted by injury or disease, no restitution of function can take place until new fibres grow from the central stumps to reach the denervated end-organs. The principal interest was then in the pattern of nerve degeneration and in the outgrowth of new axons from the site of the lesion. Of recent years, however, it has become clear that the nervous system possesses power additional to that normally understood by regeneration. This is the phenomenon known as sprouting whereby remaining intact nerve fibres, in response to a stimulus from adjacent degenerating fibres, give off fine branches which come into contact with the adventitial cells left by the degenerating axons and are thereby guided to denervated structures. In this situation, neighbouring undamaged nerve fibres are playing an active part in recovery, locally readapting anatomical and physiological relations so as to modify the abnormality of function after damage to nervous tissue. This mechanism, called collateral nerve sprouting, was first established in skin partly deprived of its sensory nerve supply (Weddell, Guttmann & Gutmann, 1941). It has been extended to muscle incompletely denervated of its motor supply (see Edds, 1953), but only recently has direct evidence been obtained of its occurrence in the autonomic nervous system (Murray & Thompson, 1956, 1957). It should be noted, however, that a remarkable recovery of function after partial denervation in the sympathetic nervous system was described many years ago (Simeone, Cannon & Rosenblueth, 1938; Geohegan & Aidar, 1942), and since then several other pointers to a return of orderly or bizarre function, quicker than could be explained by any classical process of regeneration, have come to notice.

In the following account, an outline will be given of the experiments establishing the existence of this type of sprouting in the autonomic nervous system; some of the characteristics of the sprouting process will be outlined; finally the possible implications of sprouting for certain physiological and clinical situations will be examined.

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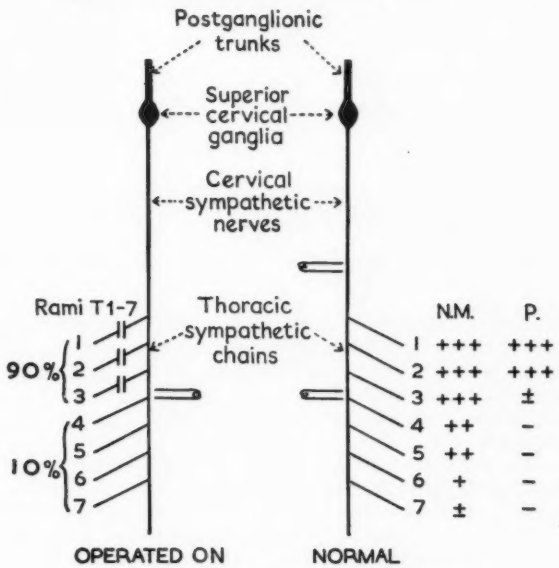
1. Sprouting in the Sympathetic Nervous System

The superior cervical ganglion of the cat is innervated preganglionically by contributions from seven thoracic rami (T1–7) (fig. 1). Of these, T1–3 contribute about 90% of the preganglionic fibres, the remaining 10% coming from T4–7. If T1–3 rami are sectioned, there is left, therefore, only 10% of the original preganglionic innervation. If stimulation of rami T4–7 is then done immediately, it produces only a very small contraction of the nictitating membrane (this membrane is conveniently used as a sign of activity in the superior cervical ganglion). But if the test is repeated 4–8 weeks later, the response to stimulation of the intact 10% of fibres arising from T4–7 is very much increased; indeed it equals the response obtained by stimulating the whole of the cervical sympathetic nerve on the normal side (figs. 2, 3). Thus a process has taken place whereby, over a period of weeks, 10% of the preganglionic fibres have taken over the function of the normal total innervation. A second feature is also important; normally stimulation of the rami below T3 elicits no, or trivial, dilatation of the pupil, since the pupillodilator fibres arise mostly from T1 and T2, and to a less extent from T3 (fig. 1). But several weeks after the partial denervation, the excitation of the rami below T3 now produces a

FIG. 1. DIAGRAM OF THE EXPERIMENTAL LESIONS OF THE NERVES, THE LEVELS AT WHICH THEY WERE STIMULATED, AND THE ORIGINS OF SOME OF THE PREGANGLIONIC FIBRES



N.M.: contraction of nictitating membrane
P.: dilatation of pupil

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FIG. 2. RESPONSES OF NICTITATING MEMBRANES AND SUPERIOR CERVICAL GANGLION TO STIMULATION OF RAMI

Upper records: response of nictitating membrane

a: on side operated upon, on which rami T1–3 had been divided 49 days previously

b: on normal side

(Rami T4–7 on both sides stimulated simultaneously)

Middle records: contractions of nictitating membranes in response to intravenous injections

c: adrenaline (15 μg.) on side operated upon

d: noradrenaline (100 μg.) on side operated upon

e: adrenaline (15 μg.) on normal side

f: noradrenaline (100 μg.) on normal side

Bottom records: action potentials recorded from superior cervical ganglion

g: partially denervated ganglion, T1–3 cut 70 days previously; rami T4–7 stimulated, showing action potential of a magnitude comparable with that obtained on stimulation of entire cervical sympathetic nerve on normal side (at j)

h: normal ganglion, T4–7 only stimulated

j: normal ganglion, whole of cervical sympathetic nerve stimulated

All records at same amplification.

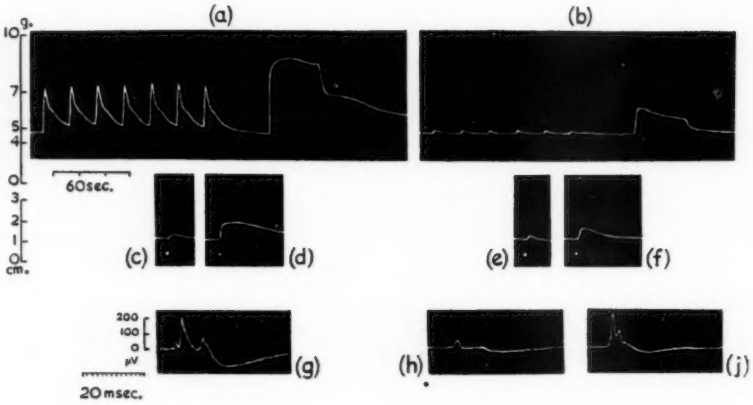


FIG. 3. SIMULTANEOUSLY RECORDED RESPONSES OF NICTITATING MEMBRANES

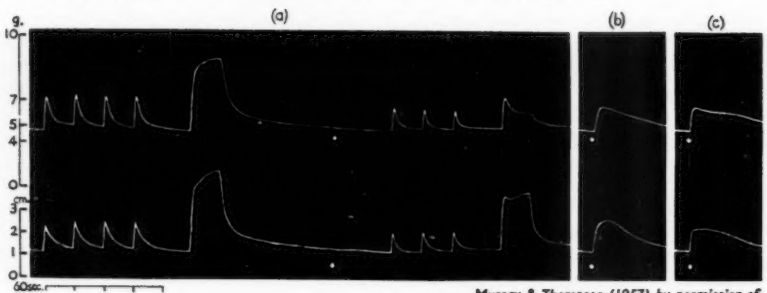
Upper record: response on side operated upon (Rami T1–3 divided 28 days previously)

Lower record: response on normal side

a: stimulation of T4–7 on side operated upon and of cervical sympathetic nerve on normal side. Intravenous hexamethonium (1 mg./kg.) then given, followed by further stimulation

b: intravenous adrenaline (25 μg.)

c: intravenous noradrenaline (100 μg.)



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The smaller responses of the nictitating membrane to nerve stimulation are due to 4 supramaximal stimuli at 1 cyc./sec. repeated every min., and the larger at 10 cyc./sec. continuously for 1 or 2 min. Injections are indicated by white spots.

Scales in g.: tensions exerted Scales in cm.: excursion of lever (magnification 1 : 6.5) Scale in μv: ganglion action potentials

vigorous pupillary dilatation (fig. 4). This shows that the recovery process, in addition to restoring function as assessed by the contraction of the nictitating membrane, has also been such that the preganglionic fibres remaining now make connexions with ganglion cells not previously activated by them.

The return of function might be attributed to a developing hypersensitivity of the nictitating membrane or of the ganglion cells, of the kind well established following denervation. It is true that hypersensitivity is present in the earlier stages, but four weeks or longer after operation this hypersensitivity has disappeared and yet the recovery of function is fully maintained. Indeed, the disappearance of the hypersensitivity appears to coincide with re-innervation by collateral sprouts and the consequent full functional recovery.

a. Histology

Histological examination of partially denervated ganglia from an experiment such as that just described shows fine nerve branches arising from the remaining intact preganglionic fibres, particularly from the peripheral terminations within the ganglia (Plate II, figs. A, B, C & D). These tiny sprouts are detectable within five days of operation, but they continue to mature for at least 1–2 months. The sprouts can be seen to come into close apposition with most of the ganglion cells in the ganglion, particularly with their dendritic processes.

b. Functional Efficiency of the Partially Denervated Ganglion after Sprouting

If electrical records are taken from the ganglion (fig. 2), it can be shown that the majority of the cells within the partially

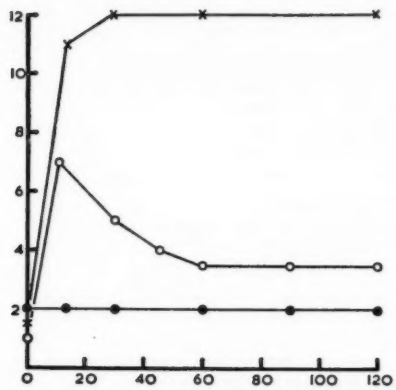
denervated ganglion are now activated by stimulation of the remaining 10% of preganglionic fibres. Although differences in the ganglion action potential are detectable, these could be attributed to the conduction time in the remaining preganglionic fibres which have sprouted. It can be shown, too, that the acetylcholine output from the preganglionic endings has been considerably increased, as compared with that normally released by this fraction of the normal preganglionic trunk; the output after sprouting is about twice as high as that normally coming from these fibres. On the other hand, the recovery is not such as to return the ganglion to the normal state. For instance, the amount of acetylcholine released in such a ganglion is only about 60% of that obtained from stimulating the whole preganglionic trunk, i.e., the cervical sympathetic nerve to the normal ganglion. Further, the partly denervated ganglion becomes more sensitive to ganglion-blocking agents than a normal one (fig. 3).

The evidence described above shows that preganglionic fibres are capable of sprouting in response to partial denervation. So far as it has been tested, the same appears to be true of postganglionic fibres when a trunk is partially sectioned.

2. Time-Course of the Sprouting Process

The extent and the course of recovery resulting from collateral sprouting depend on a number of factors. One of them is the number of normal intact fibres left which are available for sprouting. With 10% of the preganglionic fibres remaining intact, the stages of recovery can be conveniently described as early, intermediate and late (fig. 5). In the early stage the sensitivity of the nictitating membrane to noradrenaline is still normal, the effect of preganglionic excitation of the remaining fibres is still much reduced, and pupillary dilatation is slight. In the intermediate stage, hypersensitivity of the nictitating membrane is at its highest, the contraction of the nictitating membrane to preganglionic excitation is now becoming not only equal to the normal but actually exceeds it; and pupillary dilatation is beginning to appear. Finally, in the late, or what can be regarded as the mature stage, the contraction of the nictitating membrane is approximately normal, hypersensitivity has disappeared and the development of pupillary function is more or less complete. This sequence

FIG. 4. SIZE OF PUPIL AT DIFFERENT TIMES AFTER OPERATION, ON CONTINUOUS STIMULATION OF RAMI



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Abscissae: time (sec.)

Ordinates: size of pupil (mm.)

Rami T4–7 continuously stimulated at 10 cyc./sec.

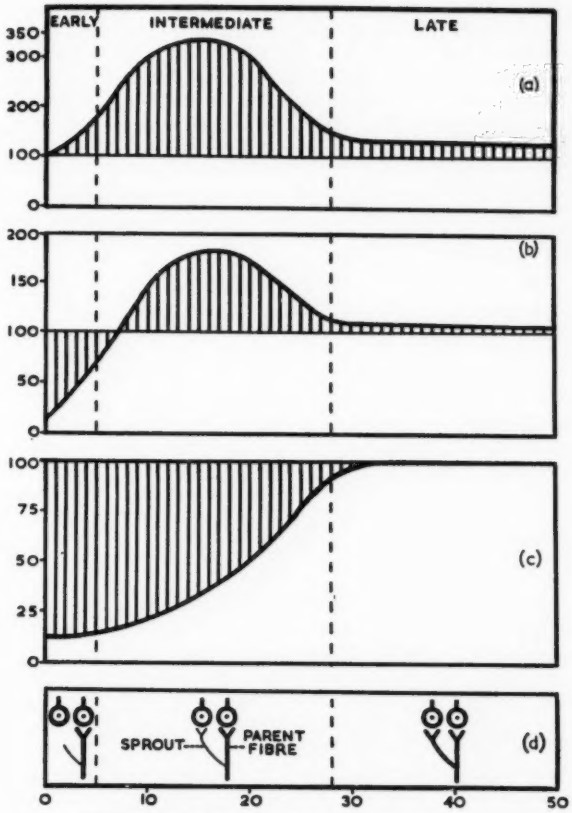
Rami T1–3 divided:

●——● 5 days previously

O——O 18 days previously

X——X 28 days previously

FIG. 5. STAGES OF RECOVERY RESULTING FROM COLLATERAL SPROUTING



Abscissae: days after operation

Ordinates: response on side operated upon expressed as percentage of that on normal side

a: sensitivity of nictitating membrane to intravenous noradrenaline (100 μg.)

b: response of nictitating membrane to preganglionic nerve stimulation

c: pupillary dilatation on preganglionic nerve stimulation

d: the sprouting process, at varying times after division of 90% of the preganglionic fibres to the superior cervical ganglion (rami T1–3 divided)

In (b) and (c) the response to stimulating the remaining 10% of fibres on the side operated upon is expressed as a percentage of that obtained on stimulating the whole of the cervical sympathetic nerve on the normal side.

can be readily interpreted as follows. While the nictitating membrane is partly denervated, hypersensitivity will increase. But as the nictitating membrane becomes re-innervated, hypersensitivity will disappear again. The disappearance, therefore, signals the full re-innervation of the membrane; and this is borne out by the coincidence of time of the return of pupillary dilatation with that of the disappearance of the supersensitivity. The exaggerated response of the nictitating membrane in the intermediate stage can be taken to represent the effect of a partial re-innervation combined with a supersensitivity still remaining, to produce an abnormally high response.

If fewer fibres are left intact, e.g. 1% or less, then the re-innervation of ganglion cells is more limited. Hypersensitivity now persists, but the responses of the nictitating membrane have always been found to remain less than on the normal side, even several months after operation, despite a substantial recovery of function compared to the original response. It was interesting to discover, however, that with an intermediate number of fibres left intact, the paradoxical situation arose whereby the response after sprouting attained and remained at a greater level than the normal; this stage has persisted for at least ten months. Here the re-innervation is never complete, although it becomes substantial; thus hypersensitivity can persist; and the summation of these two factors can allow an exaggerated response.

3. The Stimulus to Sprouting

Sprouting is a response by residual intact fibres to the presence of adjacent degenerating fibres. The stimulus is thought to be the release of a humoral agent by the degenerating fibres (Edds, 1953). Localized disorganization of the axolemma is followed by the outgrowth of fine axoplasmic strands which penetrate the nerve fibre sheath and establish contact with adventitial cells, i.e., Schwann cells in peripheral nerves or their homologues, gliocytes in ganglia. These adventitial cells guide the sprouts so that they may eventually reach effector organs. It is thought that the chemical stimulus is readily diffusible, since it may act at a considerable distance from its source (Hoffman, 1951). Whatever the stimulus, it is to be expected that the greater the intermingling of degenerating and intact fibres, the greater the number of new sprouts formed. This probably explains why sprouting is so vigorous and effective in an autonomic ganglion in which there is a very dense collection of nerve cells and fibres. However, if the partial denervation is very extensive so that only a few intact fibres are left for sprouting, the recovery of function may then be relatively restricted. As regards the actual nature of the stimulus, it is still unidentified, although there are known to be a number of specific chemical substances able to promote or retard the sprouting process (Edds, 1953; Guth, 1956).

It seems probable that the fine terminations of nerve fibres are more sensitive to the stimulus for sprouting (Edds, 1953). But collateral sprouting certainly occurs at more proximal levels in the main nerve trunk (Causey & Hoffman, 1955; Murray & Thompson, 1957). There may, however, be some adaptive character in the process; sprouts occurring at terminations will have a greater chance of reaching suitable end-organs, of developing successful functional unions and consequently maturing (Weiss, 1941; Aitken, Sharman & Young, 1947; Evans & Murray, 1956). It is not known whether sprouts which occur at more proximal levels, and which may fail to reach end-organs, undergo resorption or remain small and ineffective.

4. Factors Controlling the Effectiveness of Sprouting

The outstanding feature of the sympathetic nervous system, in contrast to the somatic innervation of skeletal muscles, is the remarkably complete return of function after partial denervation, even when there are only a few fibres available for sprouting. At least three factors probably contribute to this: (i) For sprouts to function, for instance in a limb muscle, they must establish contact with denervated end-plates; this depends on the availability of Schwann sheaths to guide growing sprouts to the end-plates (Morris, 1953). Because of the compact nature of the ganglion, there will be an extensive intermingling of intact and degenerating fibres. Further, in contrast to muscle in which the Schwann sheaths leading to motor end-plates are relatively discrete, the adventitial cells within the ganglion that guide regenerating nerve fibres to ganglion cells form a syncytium (De Castro, 1951; Causey & Hoffman, 1955). It seems likely, therefore, that in a ganglion a large number of sprouts would be formed and that they would be more readily guided towards their appropriate end-organs. (ii) The course of the sprouts is almost certainly a good deal shorter in the ganglion than in, for instance, a striated muscle. (iii) The chance of a functional union to be made depends to some extent simply on the incidence of suitable destinations in the path of the sprouting fibre. Whereas end-plates are distributed only sparsely throughout a muscle, a ganglion is densely populated with ganglion cells. Furthermore, the target area of the ganglion cell and its dendrites for sprouts to make functional connexions may be relatively large (Murray & Thompson, 1957).

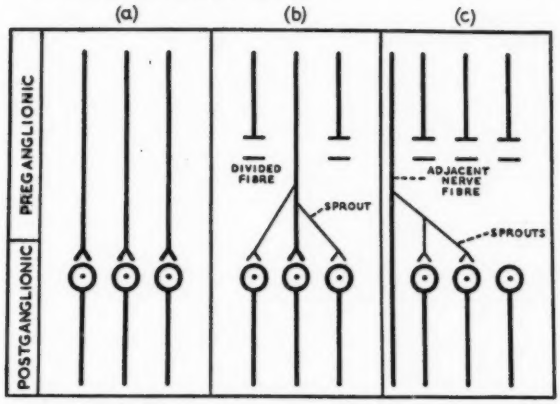
A factor which would, on the other hand, limit the innervation of the ganglion is the capacity of a parent fibre to produce the number of sprouts required. It has been calculated (Edds, 1953) that growing sprouts constitute less than 1% of the volume of the whole neurone. But the axoplasm drawn into them may be available only locally and at the expense of the axoplasm at the point of origin rather than of the whole neurone. Nerve cells which have supported new branches for some time enlarge (Cavanaugh, 1951), and their axons hypertrophy (Edds, 1949). No doubt this assists a small number of axons to support a large number of new sprouts and to allow them to mature, although the full adaptation may take many months (Edds, 1953).

It is clear, therefore, that many factors influence the degree and time-course of recovery. The number of residual fibres available for sprouting, the closeness of intermingling of degenerating and intact fibres, the distance the sprouts have to grow and the effectiveness with which they are guided to their destination, all are of major importance. In addition, the resulting recovery will also be modulated by the balance between re-innervation and remaining hypersensitivity; the evidence suggests that the overshoot of response of effector organs is more prolonged when the sprouting is postganglionic than when it occurs with preganglionic fibres (Murray & Thompson, 1957).

5. Changes in Pattern of Autonomic Innervation

After partial denervation, the main feature is recovery of function. This may show certain unexpected results. For

FIG. 6. DIAGRAM SHOWING RELATIONSHIP OF PREGANGLIONIC FIBRES IN A NORMAL AND IN DENERVATED GANGLION



a: normal ganglion

b: partially denervated ganglion. Sprouts have arisen from an intact preganglionic fibre within the ganglion to re-innervate denervated ganglion cells.

c: completely denervated ganglion. Sprouts have arisen from an adjacent fibre hitherto unrelated to the ganglion, to re-innervate denervated ganglion cells. This may lead to bizarre phenomena.

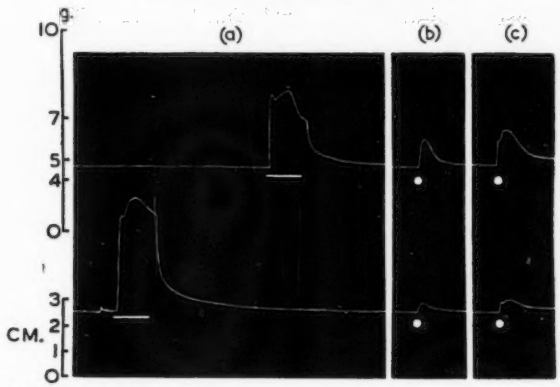
example, there has already been mentioned above the fact that preganglionic fibres normally associated exclusively with cells innervating a nictitating membrane may sprout and make functional connexion with those distributed to the pupil, and this type of re-innervation is illustrated in fig. 6b. It appears that sprouts may re-innervate any effector organs of the same type (i.e. cholinergic or adrenergic), irrespective of the original connexions of the parent fibre and end-organ. Thus, it has long been known that preganglionic cholinergic fibres can functionally re-innervate a motor end-plate, and that a motor fibre can similarly re-innervate ganglion cells (Langley & Anderson, 1904; De Castro, 1951). This re-innervation, which is illustrated diagrammatically in fig. 6c, may lead to bizarre results. In the experiment to which fig. 7 refers, complete preganglionic denervation of the superior cervical ganglion resulted in sprouting of vagal efferent fibres passing through the adjacent nodose ganglion. The sprouts then partly re-innervated the superior cervical ganglion cells by way of existing communications. As a result, nictitating membrane contractions on the side operated upon could be elicited by stimulating the contralateral vagus centrally or by eliciting gastric or vascular reflexes. This was possible, because impulses which passed up the contralateral vagus and via central connexions down the ipsilateral vagus were able to activate cells in the denervated superior cervical ganglion through new connexions which had been formed by collateral sprouts from the ipsilateral vagus.

6. Some Applications

Collateral sprouting is a general phenomenon of nervous tissue and occurs in all mammals so far tested, including man (Coërs, 1955a, 1955b). Sprouts can arise not only from sensory and motor fibres, but also, as has been shown above, in the autonomic nervous system. This occurs also in the central nervous system (in the spinal cord of cat and monkey) (Liu & Chambers, 1956; McCouch, Austin & Liu, 1956). Moreover, sprouting occurs not only in response to trauma or operation, but also to a wide variety of diseases which cause partial denervation, such as poliomyelitis, polyneuritis and progressive muscular atrophy (Coërs, 1955a, 1955b). The readiness with which it occurs in the autonomic nervous system and its wide distribution throughout all nervous tissue suggest that it may play a more extensive part in certain physiological and clinical phenomena than has hitherto been recognized. The problem of establishing that sprouting has occurred and of identifying the origin and course of such fibres is, in many instances, a formidable task. In the section which follows, it is intended to do no more than indicate some of the types of situation in which sprouting has to be seriously considered (see Table I).

Important examples in experimental physiology are the so-called pseudomotor phenomena. In 1863, Philipeaux & Vulpian showed that, after section and degeneration of the hypoglossal nerve, stimulation of the chorda-lingual on the same side will then result in a contraction of the tongue. Rogowicz (1885) observed a similar phenomenon; after section and degeneration of the facial nerve, stimulation of the cervical sympathetic on the same side caused a contraction of the facial muscles near the lips and eye. Sherrington (1894) noted that, when the motor fibres in the sciatic nerve had undergone degeneration after section of the appropriate

FIG. 7. SIMULTANEOUSLY RECORDED RESPONSES OF NICTITATING MEMBRANES



Upper record: responses of right nictitating membrane

Lower record: responses of left nictitating membrane

(Complete preganglionic denervation of the right superior cervical ganglion 109 days previously.)

a: on the right side: response to stimulation of central end of cut left vagus. On left side: response to stimulation of the cervical sympathetic nerve. Stimulation as indicated by white horizontal bars, for 2 min. at 10 cyc./sec.

b: responses to 10 μg. adrenaline intravenously

c: responses to 100 μg. noradrenaline intravenously

The moment of injection at (b) and (c) is indicated by white spots.

Responses in (b) and (c) show persisting hypersensitivity on the side operated upon.

Upper scale: tension exerted in g.

Lower scale: excursion of lever in cm. (magnification 1: 6.5)

TABLE I. SELECTED LIST OF PHENOMENA POSSIBLY INVOLVING COLLATERAL SPROUTING

Type of fibre degenerating	Type of fibre sprouting	Resulting phenomena
Motor	Motor	Recovery of function after partial denervation of motor nerve following injury or disease, e.g., pressure on nerve roots, anterior poliomyelitis, amyotrophic lateral sclerosis (Coërs, 1955a, 1955b; Woolf, 1955)
	Autonomic	Pseudomotor phenomena (see, for example, Philipeaux & Vulpian, 1863; Rogowicz, 1885; Sherrington, 1894)
Sensory	Sensory Autonomic	Post-herpetic pain
Autonomic	Autonomic	Recovery of function after sympathectomy, e.g., surgical sympathectomy of thoracicolumbar region and upper limb. Bizarre phenomena—gustatory sweating, auriculo-temporal syndrome, chorda tympani syndrome (Haxton, 1948; Young, 1956)
	Autonomic Sensory	Post-sympathectomy pain (Tracy & Cockett, 1957)
Mixed: Motor Sensory Autonomic	Sensory Autonomic ? Motor	After peripheral nerve injury, e.g., causalgia, phantom limb pain; and after disease, e.g., polyneuritis
Central	Central	Spasticity occurring after hemisection of the spinal cord (McCouch *et al.* 1956) Excessive sweating in syringomyelia (Wilson, 1936); encephalitis (Langenskiöld, 1946) Possibly after any disease causing degeneration in the central nervous system

ventral roots, stimulation of the nerve trunk caused a response of the "paralysed" muscles. In each instance, a nerve develops the ability to produce contractions in muscles which have been paralysed by section and degeneration of their motor nerves. It is generally supposed that a chemical substance, almost certainly acetylcholine (Dale & Gaddum, 1930), is liberated during stimulation of the appropriate nerves (containing cholinergic vasodilator or secretomotor fibres), which then diffuses over a distance and activates muscle rendered hypersensitive by the previous denervation (Cannon & Rosenblueth, 1949). Although acetylcholine is undoubtedly concerned, it seems likely that collateral sprouting rather than simple diffusion is required in order to explain these phenomena fully, especially in view of the fact that some of these nerves fully retain their motor effects even after re-innervation of the muscles by their normal motor supply (Philipeaux & Vulpian, 1863); for, under these conditions, there should be a considerable if not complete disappearance of the supersensitivity of the musculature. The rapid and wide-spread effects of collateral sprouting require consideration in any physiological experiments involving chronic nerve section: for example, some of the studies on antidromic vasodilatation.

Similar phenomena in clinical practice fall mainly into two groups: (i) return of reflex autonomic function after surgical sympathectomy, and (ii) bizarre phenomena. The operation of thoracicolumbar sympathectomy for hypertension is known to leave a relatively large number of intact fibres responsible for the so-called "escape" areas (Boyd & Monro, 1949; Boyd, 1957). Return of reflex function often occurs within a few weeks of operation, and the work of Monro (1954) points to sprouting as the mechanism responsible. Preganglionic sympathectomy for the upper limb leaves, at least, the first ramus as a source of intact fibres available for sprouting (Geohegan & Aidar, 1942). Even after ganglionectomy, possible escape routes exist in the intermediate ganglia and their aberrant preganglionic and postganglionic fibres (Skoog, 1947; Boyd & Monro, 1949; Boyd, 1957).

An example of a bizarre phenomenon in man might well be gustatory sweating. This sometimes occurs after local trauma or disease in the auriculo-temporal or submental regions or after sympathectomy for the upper arm (Haxton, 1948; Young, 1956). It consists of sweating, accompanied by flushing, in the head and face in response to the stimulus of taste. The localized form of the disorder could be due to sprouting of cholinergic fibres, for example, secretomotor, in response to degeneration of adjacent fibres which normally supply the sweat glands and blood vessels of the face. When the syndrome occurs after sympathectomy for the upper limb, re-innervation of the superior cervical ganglion could be by sprouting from residual preganglionic fibres, or from adjacent efferent vagal fibres. Exaggerated reflex responses may be due to the summation of the effects of partial re-innervation by sprouts and persisting hypersensitivity of sweat glands (Wilson, 1936).

Pain commonly follows damage to peripheral nerve trunks by injury or disease. Causalgia is one of the best-known examples. In such circumstances, sprouting will involve motor, sensory and autonomic fibres not only at and distal to the site of lesion, but for some distance proximal (Evans & Murray, 1956). Indeed, since a proportion of nerve fibres degenerate centrally as far as their cells (Ranson, 1947), sprouting can occur as far proximally as the central nervous system. In any regenerative process, sprouts may lie in very close apposition to one another so that their axoplasmic surfaces are in contact (Young, 1949; Evans & Murray, 1954); they may act as synaptic junctions. Such artificial synapses can persist, or, because of maturation or resorption of sprouts, can cease to function. The clinical features of causalgia and the satisfactory response to sympathectomy have been explained on the hypothesis that such artificial synapses are formed, so that impulses in efferent sympathetic fibres stimulate sensory somatic fibres both centrally and peripherally (Doupe, Cullen & Chance, 1944). The occurrence and interaction of collateral sprouts could explain many of the puzzling phenomena associated with causalgia.

Past failure to recognize the possibility of regenerative processes in the central nervous system has been largely due to inadequate technique of experimentation (Windle, 1956). The effects of sprouting in autonomic ganglia may serve as a simple model for those we may anticipate in the central nervous system.

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TRANSMISSION IN AUTONOMIC GANGLIA

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1 Phases of ganglionic transmission
2 Factors affecting transmission
3 Complicating factors in cholinergic transmission
4 Parasympathetic transmission
References

The reader of this symposium cannot fail to be struck by the multiplicity of interests involved in a study of the physiology of the autonomic system. For me, however, above and beyond the various interests inherent in the autonomic system itself, is an overriding fascination afforded by the existence, in a sympathetic ganglion, of a microcosmic nervous system. Here nature provides us with an aggregate of neurones, readily accessible to experiment, readily isolated from the influences of other active neurones, sometimes even fitted with modern plumbing in the form of a vascular system that can, with relative ease, be segregated from the general circulation. What more can the physiologist ask of nature?

It is disappointing to find how little advantage has been taken of the opportunity thus afforded. Eccles (1935a) showed us, almost at the start, what was available. Many of the subtle interactions between neurones which must form the basis for the integrative and modulatory activities of the brain are also exhibited in the microcosm of the ganglion. Here we have summation, both spatial and temporal; here we have facilitation and occlusion; here we have almost all the Sherringtonian principles of integrative action. But since Eccles first demonstrated these phenomena, they have been shamefully neglected. Neither physiologists nor pharmacologists have probed any further.

Instead we have all "shocked" the ganglion with maximal electrically-induced volleys in the presynaptic nerves at frequencies chosen only to suit ourselves. We have learned a great deal about how the ganglion cells react to such heroic treatment, and, indeed, the remainder of this article is largely devoted to describing these findings. It is all the more important to begin by warning that we are not yet ready to make inferences about the normal functioning of the microcosm of a ganglion; and, far less, are we ready to make inferences about the normal functioning of the macrocosm of the brain.

All the descriptive data in this paper refer, unless otherwise stated, to the superior cervical ganglion of the cat, which has, for reasons of experimental simplicity, been the preparation of choice in most of the studies of ganglion physiology. I have first tried to describe the normal events taking place in the ganglion during the transmission of nerve impulses. The description is based on the assumptions that all the presynaptic fibres are cholinergic and that all synapse in the ganglion. Neither assumption is necessarily correct. It is just possible that non-cholinergic presynaptic fibres exist—if they do, they are a small minority of the total population of presynaptic fibres; there is certainly a number of so-called through-and-through fibres, most of which are postsynaptic fibres arising from cells proximal to the ganglion being studied.

In view of these reservations to the uncomplicated picture of normal cholinergic transmission, I have attempted, after describing the normal transmission processes, to summarize the various anomalies in this picture.

1. Phases of Ganglionic Transmission

When we apply a maximal electrical stimulus to the presynaptic nerve supplying the ganglion, we induce all the nerve fibres in the nerve to fire simultaneously. This in turn leads to the discharge of all the cells in the ganglion, although this discharge is not quite synchronous. It is not synchronous because the distance between the point of stimulation on the presynaptic nerve and the individual ganglion cells is variable; and, even more important, because the size of the presynaptic fibres and their conduction velocity is also variable. The asynchrony is not, however, sufficiently great to prevent the recording from the postsynaptic nerve of a relatively uncomplicated action potential—i.e., of an approximately synchronous discharge of all the axons in the postsynaptic nerve. Nevertheless, the slight asynchrony is important to a proper understanding of the electrical events in the ganglion.

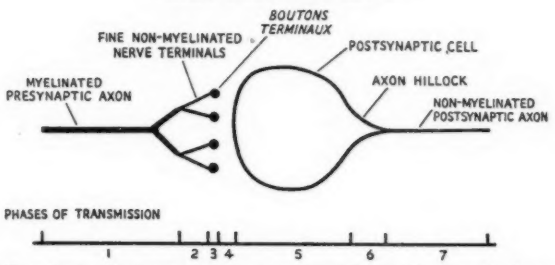
When the ganglion is stimulated in this way, it is convenient to try to follow the events during transmission in terms of a simplified model of the synapse (fig. 1). The ganglion is not in the least like the model, for nerve terminals ramify over the entire surface of the cells, and one presynaptic axon will terminate on numerous presynaptic axons. Nevertheless, for our artificial maximal stimulus the model will serve a useful purpose.

On this model the events initiated by the maximal presynaptic volley can be classified into seven distinct phases. I have represented these phases spatially along a horizontal scale, which again is an over-simplification of the true picture. The seven phases may be described as follows.

Phase 1: Saltatory Conduction in the Myelinated Presynaptic Fibre

The process of saltatory conduction in myelinated axons has received a great deal of elegant study in recent years, and

FIG. 1. DIAGRAMMATIC REPRESENTATION OF GANGLION SYNAPSE



The phases of transmission are represented spatially along the horizontal scale. (The numerals represent the phases; see section 1 of text.)

a detailed picture of the mechanisms involved is emerging. There is no reason to think that presynaptic fibres vary in any essential way from other axons of similar size in respect of the nature of the propagated action potential and there is no need to discuss the mechanisms here.

Phase 2: Conduction in the Non-Myelinated Presynaptic Terminals

It is generally believed that the propagated action potential is conducted along the branching terminals—which are not myelinated—of the presynaptic axons. During this phase of the passage of the impulse it may be subject to influences which have no role in modifying conduction in the myelinated axon proper.

Phase 3: Release of Acetylcholine at boutons terminaux

When the action potential has traversed the fine terminals of the presynaptic axon it causes, on reaching the *boutons terminaux*, the release of a quantum of acetylcholine. It has been estimated that a single maximal volley causes the release, in the superior cervical ganglion, of the cat, of about 0.1 mμg. of acetylcholine (Feldberg & Vartiainan, 1934; Perry, 1953).

Phase 4: Acetylcholine Crosses the Synaptic Gap; Occupies Receptors on the Cell Membrane; and is Eliminated

For a brief fraction of time the acetylcholine released is present free and crosses the synaptic gaps. It then, so it is presumed, forms some sort of attachment to the membrane of the ganglion cell, fixing on to the so-called receptor sites. Having occupied these receptors, the acetylcholine is then rapidly eliminated. It may be eliminated in a number of ways, from physical diffusion to specific hydrolysis by cholinesterase. It is certainly subject to hydrolysis from the moment it is released from a protected or " bound " form in the *boutons terminaux*.

Phase 5: Local Response of Cell to Acetylcholine

While the acetylcholine is attached to the receptor sites on the cell membrane and before it is eliminated, it produces a change which results in a local depolarization of the cell membrane. This depolarization is not propagated and spreads decrementally from the area immediately beneath the *boutons terminaux*. When all the boutons are discharged by a maximal volley, the local response presumably occurs simultaneously all over the cell surface (some 70% being covered by boutons (de Castro, 1942)), the local response of the cell being the sum of all local responses under individual boutons.

Phase 6: Initiation of Propagated Action Potential

When the local response of the cell exceeds a certain critical level (which may represent a critical area or a critical degree of depolarization), a propagated action potential is initiated. The site at which this initiation occurs is not known, but for the purposes of diagrammatic representation this is immaterial. The important fact is that a propagated action potential reaches the postsynaptic axon.

Phase 7: Conduction in the Non-Myelinated Postsynaptic Axon

The propagated action potential is conducted along the postsynaptic axon, and the transmission process is complete.

2. Factors Affecting Transmission

It is interesting to examine the factors affecting transmission in the light of the various phases of transmission just described. Before doing so, it is desirable to summarize the ways in which the function of each phase can be tested experimentally. This is shown in Table I. It must also be

TABLE I. EXPERIMENTAL TESTS OF THE FUNCTION OF PHASES OF TRANSMISSION

Phase of transmission	Test to ensure this phase is functioning normally (Each test applies only if the preceding tests listed in this table have been carried out successfully.)
1	Stimulate presynaptic nerve and record action potential at a point distal on the same nerve.
2 and 3	Stimulate presynaptic nerve to eserinized perfused ganglion and test effluent for presence of acetylcholine. (It is not yet possible to differentiate with certainty between Phases 2 and 3.)
4 and 5	Stimulate presynaptic nerve and record local potential of ganglion cell. (It is not yet possible to differentiate between Phases 4 and 5.)
6	Stimulate presynaptic nerve and record propagated action potential in postsynaptic nerve.
7	Stimulate postsynaptic nerve and record action potential at a point distal on the same nerve.

remembered that a factor may affect any one phase of transmission, not only directly but also indirectly, by interfering with a function which subserves that particular phase. Thus depression of the release of acetylcholine may be direct or may be indirect as, for example, the result of a depression of acetylcholine synthesis; or initiation of the propagated action potential may be prevented by direct action on the cell membrane or indirectly by depression of the cell metabolism.

Factors Affecting Phase 1

There is no point in discussing here the many factors known to affect saltatory conduction, since this has recently been reviewed by Stämpfli (1954). It is, however, worth mentioning that, pharmacologically, local anaesthetics such as cocaine are powerful agents for artificially interrupting saltatory conduction; and there is frequently a need, in the analysis of a new drug, to exclude such activity before presuming it to possess true ganglion-blocking potency.

Factors Affecting Phase 2

It is difficult to separate by any experimental technique the effects on transmission occurring at Phase 2 from those occurring at Phase 3. Nevertheless, the action of the toxin of *Cl. botulinum* deserves mention at this point. Ambache (1949, 1951) showed that the toxin possessed a characteristic action in blocking transmission at cholinergic junctions, namely, at motor end-plates and at ganglion synapses. He showed that presynaptic stimulation failed to excite the end-organ but that acetylcholine would excite the postsynaptic cell; he furthermore showed that Phase 1 was not affected by the toxin. He therefore inferred that block occurred at Phase 2 or at Phase 3. Burgen, Dickens & Zatman (1949) showed directly that the toxin prevented the release of acetyl-

choline at the neuromuscular junction. They discussed three possible mechanisms, namely, block of Phase 2, block of Phase 3, or indirect block of Phase 3 due to depression of acetylcholine synthesis, and came to the conclusion that Phase 2 block, due to an irreversible fixation of the toxin on the fine non-myelinated fibres, was the most probable explanation. This view received some support from Brooks (1954), who was able to release acetylcholine by massive repetitive stimulation even after blocking with botulinum toxin. On the other hand, Brooks (1956), arguing by analogy from the work of Castillo & Katz (1955), put forward the considered view that the action occurred at the sites of acetylcholine release, the tips of the nerve fibres, i.e. by an action on Phase 3. Thus the difficulty of differentiating between actions at the two sites still defeats any attempt to define with certainty the site of action of botulinum toxin.

Factors Affecting Phase 3

The precise mechanism of the release of acetylcholine at the *boutons terminaux* remains obscure, in spite of the very large amount of work done. In another contribution to this volume, Birks & MacIntosh (1957) discuss the general problems of the storage and release of acetylcholine. The release of acetylcholine during prolonged stimulation has been studied by Perry (1953) who found that, over a wide range of frequencies of stimulation, the output fell exponentially to a final constant rate per minute. This implied that at a high frequency a single stimulus released less acetylcholine than did a single stimulus at a low frequency; and Perry argued that the explanation lay in the exhaustion of the stock of " available " acetylcholine and that synthesis failed to keep pace with release. Perry also suggested that the necessary presence of an anticholinesterase accelerated this exhaustion of the stock, since it prevented the re-synthesis of choline to acetylcholine in the presynaptic terminals. This view is not yet proved.

Other factors play a large part in determining the release of acetylcholine and it is necessary to examine some of them at this point.

i. *Inorganic cations.* Brown & Feldberg (1936) showed that perfusion of the ganglion with Locke's solution containing a raised proportion of potassium resulted in a prolonged release of acetylcholine. It has been a favourite hypothesis that this finding is an illustration of the normal mechanism of acetylcholine release, since potassium certainly must leak out of the nerve terminals during passage of the action potential. Harvey & MacIntosh (1940) showed, however, that this release by potassium would not occur in the absence of calcium ions. Recently, Fatt & Katz (1952) suggested that the release of acetylcholine at the neuromuscular junction might occur, in consequence of the entry of sodium ions, by a cation exchange mechanism. Hutter & Kostial (1955) showed, however, that reduction of the sodium-ion concentration does not affect the release of acetylcholine at the ganglion until it reaches a level which prevents saltatory conduction in the presynaptic axons (Phase 1). Other experiments on the role of calcium and magnesium ions were reported by Hutter & Kostial (1954). The effect of these various cations on acetylcholine release is summarized in Table II. It is apparent that release of acetylcholine is dependent upon a proper balance between these cations; but the precise nature of the control is not yet clear.

ii. *pH and temperature.* MacIntosh & Emmelin (1956) showed that the variation in pH resulting from changing from phosphate-buffered to bicarbonate-buffered Locke's solution did not affect the acetylcholine release from the ganglion. In 1954 Brown showed that the release of acetylcholine was susceptible to fluctuations in temperature. Reducing the temperature from 39° C. to 20° C. reduced the acetylcholine output to approximately one-tenth, although transmission continued apparently normally, as judged by the contraction of the nictitating membrane. Brown concluded that this was a clear indication of the high " safety factor " in transmission to which he had previously called attention (Brown & Feldberg, 1936). Clearly minor modifications of the amount of acetylcholine released would not, in such circumstances, have any profound effect on transmission. Yet it is important to recall, once again, that maximal presynaptic stimulation is not a normal physiological event. In this connexion it is interesting that Kostial & Vouk (1956), stimulating in the same way but at a frequency of 2 per second instead of the 10 per second used by Brown, failed to find any modification of the amount of acetylcholine released on changing the temperature from 39° C. to 20° C. Kostial and Vouk inferred that it was the synthesis of acetylcholine rather than its release which was sensitive to temperature.

TABLE II. EFFECT OF CATIONS ON RELEASE OF ACETYLCHOLINE

Cations		Acetylcholine release	Notes
Increased	Decreased		
K	—	increased	Spontaneous release occurs
—	K	normal	—
Ca	—	increased	No spontaneous release, but increase in release during stimulation
—	Ca	decreased	—
Mg	—	decreased	—
—	Na	normal	—
K	Ca	normal	Increased K has no effect in the absence of Ca
Mg + Ca	—	normal	—
—	K + Ca	normal	Decreased Ca has no effect in the absence of K

iii. *Factors affecting acetylcholine synthesis.* The release of acetylcholine in normal fashion is obviously dependent upon the pre-existence of an adequate stock of " available " acetylcholine. This stock is maintained enzymically by choline acetylase. Factors affecting synthesis, however, affect transmission only indirectly and usually only after a relatively long latency, during which the stock is depleted.

Factors Affecting Phase 4

It will be convenient to regard Phase 4 as having three separate components which we shall call a, b and c. Phase 4a represents the transient free existence of acetylcholine in the synaptic gaps; Phase 4b the stage—also transient—during which the acetylcholine is attached to the receptor sites; and Phase 4c the phase of elimination of the acetylcholine.

i. *Phase 4a.* It is probable that this represents a theoretical rather than a real stage of transmission. Nevertheless this stage, transient as it is, represents the situation, arising as a

result of nerve stimulation, which is analogous to the injection of acetylcholine into the arterial supply to a ganglion. MacIntosh & Emmelin (1956) have made an interesting comparison of the relative doses required to produce ganglionic stimulation in each case. The ratio between these doses lies between 1:10 and 1:250, but, as MacIntosh and Emmelin point out, the acetylcholine released by stimulation is distributed, not throughout the whole volume of the ganglion, but only in the "effective synaptic space". It is difficult to imagine that this space occupies $\frac{1}{250}$ the volume of the ganglion, far less $\frac{1}{10}$ of the volume. Consequently, the discrepancy is more apparent than real. To affect this stage of transmission would require a factor which altered the amount or the nature of the acetylcholine during its free existence. No such factor is known, save cholinesterase. Since cholinesterase acts throughout all stages of Phase 4, we will consider it later.

ii. *Phase 4b.* Attachment of acetylcholine to "receptor sites" is, by repute, influenced by a large number of drugs, including all those usually described as "competitive" ganglion-blocking drugs (Paton & Perry, 1953). Such drugs are supposed to act by competition with acetylcholine for the receptors. The evidence in favour of this concept is not good for the ganglion; but it is fairly good for the same drugs acting on the neuromuscular junction, so that argument by analogy has some validity. The evidence depends upon the fact that constant proportions of inhibitor and activator exert a constant effect. Such relationships hold very accurately with enzyme inhibitors in vitro and approximately with competitive blocking drugs at the neuromuscular junction. Results suggestive of a similar type of action at the ganglion have been obtained. Among the commonly used drugs reported to act in this way are tubocurarine, the methonium compounds and tetra-ethyl ammonium (Paton & Perry, 1953).

iii. *Phase 4c.* Very rapid elimination of acetylcholine after its attachment to the receptors is a vital part of efficient ganglionic transmission. It is generally attributed mainly to the action of the enzyme cholinesterase, which is known to be present in the ganglion (Sawyer & Hollinshead, 1945). Yet anticholinesterases do not so strikingly potentiate the actions of acetylcholine in the ganglion as they do at the neuromuscular junction (Eccles, 1944). Although cholinesterase undoubtedly plays a part, there is probably another mechanism involved and the nature of this has been reviewed by MacIntosh & Emmelin (1956). They suggest that the most likely mechanism is the physical removal of the acetylcholine by simple diffusion out of the limited "effective synaptic space", possibly reinforced by an increased mobility of the acetylcholine ion.

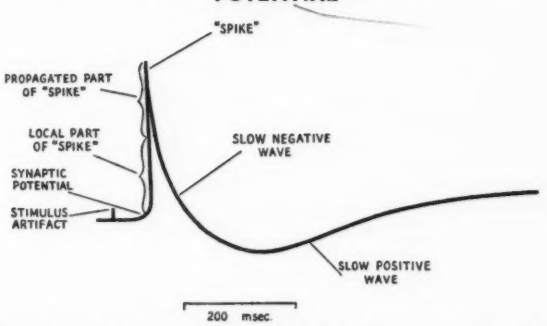
If acetylcholine were to persist for long in high concentration it would rapidly lead to a failure of transmission. This is clearly indicated by the experiments of Paton & Perry (1953) who showed that injected acetylcholine produced a long-lasting depolarization of the cell membrane, during which transmission was blocked. This failure to eliminate acetylcholine, which we have regarded as part of Phase 4, does in fact lead to block of Phase 5, namely, of the local response of the ganglion cell, owing to the prolongation of the normal effect of the transmitter substance. Thus efficient elimination of acetylcholine is essential, and, in fact, elimination is extremely efficient whether the major role is played by cholinesterase or not. The safety factor of this phase is probably much higher than that for Phase 3.

Factors Affecting Phase 5

Nature of local response. Before discussing factors which affect the local response, it is necessary to determine the nature of the response itself. The local response of the ganglion cell is a phenomenon which is, as yet, still not fully understood. If by local response we include all electrical changes which are not propagated but which spread only decrementally, then there are several separate components involved. Figure 2 shows a diagrammatic representation of the ganglionic potential complex. Apart from some 50% of the initial "spike", all the components of this complex decay exponentially, with approximately the same space constant, as they spread along the postsynaptic axon (Eccles, 1935b; Paton & Perry, 1953).

i. "*Synaptic*" *potentials.* Eccles (1937) first described what he called the detonator response of ganglion cells, but the evidence for such responses was more an inference than a clear-cut demonstration. It is extremely difficult to spot this phase in fig. 2, in normal recordings from the ganglion. Indeed Paton & Perry (unpublished data, 1952) failed to find convincing evidence for a synaptic potential of this nature even by using blocking drugs such as tubocurarine, which is the classical tool for showing the end-plate potential of

FIG. 2. DIAGRAM OF GANGLIONIC ACTION POTENTIAL



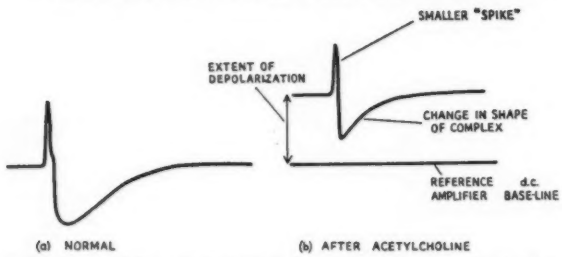
Apart from the propagated part of the "spike", the entire complex is a local response of the ganglion cell. It is recorded by a d.c. amplifier between leads placed on the ganglion body and on the cut end of the postsynaptic nerve.

muscle. They worked on the cat's superior cervical ganglion in situ. R. Eccles (1952a, 1952b), in the same ganglion from rabbits, but excised and maintained in vitro, demonstrated, using curare, changes in the complex which, she believed, indicated that the propagated "spike" had been abolished and that the remaining potential was a pure synaptic potential. In a further paper (R. Eccles, 1956) she obtained similar records with dihydro-β-erythroidine; but failed to detect any synaptic potential when using nicotine as the blocking agent.

ii. *Action of injected acetylcholine.* Paton & Perry (1953) gave intra-arterial injections of acetylcholine and recorded the changes in the resting and evoked potentials of the ganglion. Acetylcholine produced a long-lasting depolarization of the ganglion cells, a depolarization which often exceeded in magnitude the initial "spike" potential. Even if we accept the "spike" as a propagated action potential, it is not surprising that the depolarization should apparently exceed it in magnitude; the "spike" will be greatly reduced in size

owing to both temporal and spatial dispersion and to the shunting action of the tissues surrounding the cells; and more prolonged changes of potential will not be so greatly attenuated. The depolarizing action of acetylcholine did not affect the presynaptic fibres, and the effect on the postsynaptic fibres was restricted to that which would be expected from the decremental spread of a change occurring locally in the cells. These results were confirmed by Pascoe (1956) who did not, however, obtain a depolarization by acetylcholine greater than some 50% of the "spike" potential. There seems, then, to be little doubt that acetylcholine does produce a long-lasting negativity of the ganglion cells. This change is, however, also accompanied by striking alterations in the shape of the evoked potential complex. The spike height is reduced and the slow waves are greatly modified in a way which can be explained (Paton & Perry, 1953) on the assumption that the time constant of the slow negative wave is reduced almost to vanishing point (fig. 3). Eccles (1935b) had previously shown that these slow waves were also local responses of the ganglion cell.

FIG. 3. DIAGRAM OF THE EFFECT OF ACETYLCHOLINE ON GANGLIONIC ACTION POTENTIAL



Action potentials recorded as in fig. 2: (a) before, and (b) after intra-arterial injection of acetylcholine into the ganglion.

iii. *Intracellular records.* R. Eccles (1955) succeeded in recording from ganglion cells with intracellular microelectrodes. Prodromal stimulation evoked a complex described by Eccles as consisting of a synaptic potential (15–30 mv), which triggered a "spike" potential (10–100 mv). Slow "after potentials" were occasionally seen but were often obscured because of the cell injury caused by the microelectrode insertion. Antidromic stimulation never evoked any slow negative potential (R. Eccles, 1956), and it was suggested that part at least of this wave was due to the continuing synaptic potential.

It might be useful, in trying to visualize events at the ganglion cell, to consider the model of the neuromuscular junction, about which much more is known. In doing this it is, of course, essential to remember that the spatial relations at the two sites are entirely different. In the muscle cell there is a real differentiation between the membrane of the end-plate and the membrane of the remaining cell surface, so that the end-plate membrane subserves the fundamental processes underlying a local response, and the remaining membrane the fundamental processes underlying a propagated response. What is the position in the ganglion cell? Is there a similar differentiation of the membrane or do the molecular configurations subserving the two functions co-exist in a lattice structure in the same membrane?

If there is a differentiation analogous to the situation in muscle, it is very difficult to imagine that it is between membrane underlying the *boutons terminaux* and other membrane, since de Castro (1942) found that some 70% of the cell surface was covered by boutons. The only real basis for differentiation would lie in regarding the entire cell soma as being analogous to the end-plate, with the corollary that the propagated potential was initiated only in the axon. This concept is not at variance with any of the known facts. Furthermore, it receives some support from the failure of antidromic stimulation to invade the soma of the cell. This failure is illustrated by the finding of R. Eccles (1956) that intracellular electrodes failed to record any negative after-potential when antidromic stimuli were applied. It also would explain why Emmelin, MacIntosh & Perry (1949) were unable to provoke potassium loss from the ganglion by antidromic stimulation, although such loss did occur on prodromic stimulation. It is, of course, true that failure to excite by antidromic volleys might be attributable to a "safety factor" due to the sudden spatial enlargement of the post-synaptic axon at the axon hillock, so that evidence of this kind is by no means conclusive.

On the other hand the membrane of the soma may be undifferentiated except at the molecular level, and the functions of local and propagated response may co-exist. In this case it is necessary to assume that the local response is actively maintained for a relatively long time, i.e., that acetylcholine continues to exert its action throughout the whole period of "spike" potential and continues thereafter to provide the cause of the negative after-potential. Once again there is no reason why this should not be so. Thus it is apparent that the precise nature of the local response is still somewhat obscure.

Factors affecting the local response. The local response is a graded response and is thus open to all sorts of modulating stimuli. The propagated response is an all-or-nothing reaction. In consequence, it can be blocked but not otherwise modified. Most pharmacological analysis has, of course, been based on the records made of end-organ responses—in other words on the changes induced in the whole ganglion—so that block of the all-or-nothing response in a varying number of individual units also appears as a graded phenomenon. Thus the detailed mechanisms of action are often unknown, and either Phase 5 or Phase 6 may be involved.

i. *Depolarizing blocking drugs.* A group of substances, described by Paton & Perry (1953) as depolarizing blocking drugs, affect the local response of the ganglion cell to acetylcholine by reducing the normal resting potential of ganglion cells for a prolonged period of time. During this time further depolarization cannot occur and consequently transmission is blocked. The initial depolarization produced by these drugs causes repetitive firing of the ganglion cells, indicated by activation of the end-organ, but this phase may be followed by inexcitability of the ganglion cells. Nicotine and tetramethylammonium both have an action of this kind. It is not known whether these drugs also compete with acetylcholine for receptors, or whether this action on the membrane is produced at some other site or by some other mechanism. If they compete with acetylcholine they could also, of course, be regarded as agents affecting Phase 4. The depolarizing action of nicotine and tetramethylammonium was confirmed by Pascoe (1956).

ii. *Drugs modifying ganglionic transmission.* Adrenaline

(Marrazzi, 1939), histamine (Konzett, 1952) and cardiac glycosides (Konzett & Rothlin, 1952) will all, under suitable conditions, potentiate the action of acetylcholine or the effects of submaximal stimulation of the ganglion. It seems probable that such actions must take place at Phase 5. Adrenaline also possesses a blocking action (Bülbring, 1944), part of which is due to depression of acetylcholine release (Paton & Thompson, 1953). The sensitizing effect of cardiac glycosides does not occur in the absence of potassium (Perry & Reinert, 1954a); this is compatible with the idea that Phase 5 is affected; on the other hand, Perry & Reinert (1954b) have shown that absence of potassium leads to profound metabolic changes, and the effect on the cell membrane may thus be indirect rather than direct. Trendelenburg (1954, 1955) has found that the potentiating action of histamine is not blocked by competitive blocking drugs (Phase 4) and is therefore, presumably, an effect on a later phase of transmission (Phase 5).

iii. *Axotomy.* Transmission fails in a ganglion after section of the postsynaptic axon (Brown & Pascoe, 1954). Acetylcholine release occurs normally (Brown & Pascoe, 1954) and block is not due to accumulation of acetylcholine (McLennan, 1954). The failure is therefore probably in Phase 5, and Brown & Pascoe (1954) were indeed able to show that the cells of the axotomized ganglion are less sensitive than usual to acetylcholine. The explanation is not clear.

iv. *Denervation.* The extraordinary increase in the sensitivity of denervated tissues to the normal transmitter is well known and this increase does occur—although not to a very large degree—in the ganglion (Cannon & Rosenblueth, 1949). There is again little doubt that this phenomenon affects Phase 5, but the mechanism is equally obscure. Thus the presence of an intact presynaptic nerve exerts a remarkable modulating influence on the postsynaptic membrane, and no reason is yet known. Some of the effects of denervation can be strikingly mimicked by reducing the extracellular potassium concentration (Perry & Reinert, 1954b), and these authors believe that denervation induces some fundamental change in the metabolism of the ganglion cells (Perry & Reinert, 1955), possibly by inhibition of aerobic glycolysis, and that a reduction of the extracellular potassium concentration may be the initial cause of this change.

Factors Affecting Phase 6

Factors potentiating submaximal stimulation can do so only by increasing the number of postsynaptic units firing. This can be accomplished by increasing the local effect of the acetylcholine or by reducing the threshold of the mechanism for propagation of the response so that the cells fire at a smaller value of the local response. In the one case we would classify the action as affecting Phase 5, and in the other as affecting Phase 6. The distinction can be made only by recording the local response itself and, since this is seldom undertaken, we must remain in doubt about the precise nature of these changes. On the other hand, the initiation of the propagated action potential will be dependent upon the maintenance of normal conditions at the cell membrane. This maintenance is in turn dependent upon normal cell metabolism. Both the metabolic and cell membrane conditions necessary for the initiation of a propagated response may be different from those necessary for a local response. Consequently, an entirely separate set of factors may be operative in affecting the two phases. Failure to differentiate at the present moment does not imply that differentiation is unimportant. Indeed, proper differentiation might aid considerably in solving some of the outstanding physiological problems. At the moment, however, all those factors described as affecting Phase 5 may equally well affect Phase 6.

Factors Affecting Phase 7

In general, transmission in the postsynaptic axon will be subject to the same sort of effects as is transmission in the presynaptic axon (Phase 1), although it must be remembered that the postsynaptic axon is non-medullated.

3. Complicating Factors in Cholinergic Transmission

Other Nerve Pathways

Douglas & Ritchie (1956) made a detailed study of the conduction of impulses through the rabbit's superior cervical ganglion, and found incontrovertible evidence of the existence of an accessory cervical ganglion lying proximal to the superior cervical ganglion. The postsynaptic fibres from this accessory ganglion run straight through the superior cervical ganglion. Furthermore, single presynaptic axons may innervate cells in both ganglia. There appears to be no functional distinction between cells of the two ganglia (Langley, 1894; Douglas & Ritchie, 1956). The existence of through-and-through fibres means, of course, that stimulation of the cervical sympathetic nerve, distal to the accessory ganglion, will produce an end-organ response which cannot be completely blocked even if all the synapses in the superior cervical ganglion are blocked. This fact may explain certain other anomalous facts. Thus Ambache (1952) found that botulinum toxin caused a complete block of the pupil response but never a complete block of the nictitating membrane response. This has been held to indicate the possibility of non-cholinergic presynaptic fibres, but could equally well be accounted for on the basis of through-and-through fibres. The latter explanation does not, of course, account for the complete block of the nictitating membrane response, which can be produced by other ganglion-blocking agents under exactly the same conditions.

In the inferior mesenteric ganglion of the cat (Job & Lundberg, 1952) and of the rabbit (Brown & Pascoe, 1952; McLennan & Pascoe, 1954) evidence has been found for the existence of afferent fibres from the periphery (the intestine) which will excite, presumably as part of a reflex activity, the ganglion cells. No such evidence has as yet been found in the cervical sympathetic ganglia (Douglas & Ritchie, 1956). Nevertheless, the existence of reflex excitation of ganglion cells is a further complication to the picture of normal transmission. Whether such reflex excitation is also cholinergic is not known.

Other Transmitter Substances

Mention has been made of the finding of Ambache (1952) that botulinum toxin failed to block completely the transmission of impulses to the nictitating membrane. Such a finding obviously raises the possibility of non-cholinergic synapses in the ganglion, although the nature of such an alternative transmission mechanism is wholly unknown. Kewitz & Reinert (1953) showed that, under certain circumstances, a ganglion-blocking drug could inhibit the action of acetylcholine and fail to block electrical stimulation. Perry & Reinert (1954b) showed that, when the extracellular potassium concentration was reduced, hexamethonium would block transmission but failed to block injected acetylcholine. This

anomaly can be explained in a number of ways without the necessity of concluding that non-cholinergic synapses exist; but, of course, their existence would also provide an adequate explanation of the phenomena.

The action of sympathin has also been regarded as suggestive that adrenergic synapses may be found in the ganglion, and Bülbring (1944) was able to detect minute amounts of sympathin in the effluent from a perfused ganglion during presynaptic stimulation. It is quite possible that this sympathin derives not from adrenergic presynaptic fibres but from the activation of short postsynaptic fibres innervating the blood vessels of the ganglion, since postsynaptic sympathetic fibres are normally adrenergic.

There is, therefore, still doubt as to the uniformity of the fibres in the preganglionic nerve, but so far there has been no convincing demonstration of non-cholinergic fibres, although their existence cannot be excluded; the existence of through-and-through fibres and, in certain sites, of afferent reflex fibres in ganglia is undoubted.

4. Parasympathetic Transmission

The cells of the parasympathetic system are scattered throughout the end-organ which they innervate, and only in rare instances do we find anatomically recognizable ganglia, such as are common in the sympathetic system. The ciliary ganglion represents one such rare instance, and some studies of transmission through it have been made. Whitteridge (1937) described the action potential complex which showed a resemblance to that of sympathetic ganglia, save that its duration was of the order of 150 msec. as compared with some 500 msec. in the superior cervical ganglion. Suden, Hart & Marrazzi (1952) found that the ciliary ganglion responded to adrenaline in the same way as did sympathetic ganglia. Perry & Talesnik (1953) showed that acetylcholine produced a depolarization (local response) in the ciliary ganglion similar to that produced in the superior cervical ganglion, and that the actions of a number of drugs upon it closely resembled their effects on the superior cervical ganglion (Paton & Perry, 1953). Acetylcholine also potentiated the postsynaptic spike initiated by submaximal presynaptic stimulation (Perry & Talesnik, 1953). There seems, therefore, little doubt not only that transmission in parasympathetic ganglia is cholinergic, but also that there is so little difference between the fundamental reactions involved that the search for differential blocking activity is likely to be fraught with difficulty (Perry & Wilson, 1956).

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DR H. BLASCHKO is Senior Research Officer in the Department of Pharmacology at the University of Oxford. During the winter of 1953–54 he accepted an invitation to Yale University Medical School as a Visiting Lecturer and Research Associate; some of the work described in his contribution to this Bulletin was initiated during this visit. Dr Blaschko has previously contributed an article to the Bulletin (*Brit. med. Bull.* 1953, **9**, 146) on enzymic oxidation of amines, with which a fuller biographical note was published.

PROFESSOR J. D. BOYD has been Professor of Anatomy in the University of Cambridge since 1951, and is a Fellow of Clare College. He was formerly (1938–51) Professor of Anatomy in the University of London at the London Hospital Medical College. His main interests lie in the field of human embryology, with special reference to the nervous system and the placenta. He is part author of *Human embryology* (2nd ed., 1952) and of *Textbook of human anatomy* (1956). His scientific contributions include ones that have been published in the *Journal of Anatomy* (of which he is the Editor), *Contributions to Embryology*, *Proceedings of the Zoological Society*, and the *Journal of Endocrinology*. He was Woodward Lecturer in Yale University in 1955.

DR EDITH BÜLBRING is University Demonstrator in Pharmacology at Oxford. She has worked on biological assay. Her main interests have been the sympathetic nervous system, including the medulla of the suprarenal gland, the action of adrenaline and acetylcholine on nervous and muscular activity, and synaptic transmission in the spinal cord and sympathetic ganglia. She has also been interested in the automatic rhythmic activity of various tissues, including the heart, and has, in recent years, concentrated on an investigation of the properties of intestinal smooth muscle, especially its energy requirements and its electrical activity.

DR J. H. BURN is the Professor of Pharmacology in the University of Oxford. Between 1920 and 1937 he was actively concerned in biological standardization and is author of a book on that subject (*Biological standardization*, 2nd ed., 1950). In the last twenty years he has been working in fields midway between physiology and pharmacology. Thus he has been interested in the changes in the behaviour of tissues after denervation, in the common properties of different classes of drugs, the relation of stimulation to inhibition, and the phenomenon of adrenaline reversal. He has demonstrated the antidiuretic activity of nicotine and its consequences. Recently he has studied the action of acetylcholine, as a local hormone maintaining ciliary activity and in the heart. In 1956 he gave the Abraham Flexner Lectures in Vanderbilt University, Tennessee, which have been published under the title, *Functions of autonomic transmitters* (1956); this is reviewed on p. 229 of this number of the Bulletin. He is also author of *The background of therapeutics* (1948) and of *The principles of therapeutics* (1957). Professor Burn received the honorary D.Sc. of Yale University on 10 June 1957.

SIR HENRY DALE, O.M., G.B.E., F.R.C.P., F.R.S., who writes the Introduction to this symposium, has had a distinguished career. He was formerly President of the British Council, was President of the Royal Society from 1940 to 1945, of the British Association in 1947, and of the Royal Society of Medicine from 1948 to 1950. He became Director of a Department in the National Institute for Medical Research, London, in 1914, and was Director of that Institute from 1928 until his retirement in 1942. He has received a number of medals and awards, and in 1936 shared the Nobel Prize for Physiology and Medicine, the award being made with respect to his contribution to a range of physiological knowledge, with the further developments of which most of the articles composing this number of the Bulletin are concerned. He is a Trustee of the British Museum, a Member of the Standing Commission on Museums and Galleries, and Chairman of the Wellcome Trust.

DR J. DIAMOND is now on the staff of the Department of Physiology, University College, London, where he was Sharpey Scholar 1953–56 and a Medical Research Council assistant 1952–53. He has been mainly concerned in the analysis of behaviour of pressure receptors in the carotid sinus under the influence of various drugs, and in the investigation of pharmacological properties of regenerating nerve-endings in experimentally produced nerve lesions and neuromatas. Dr Diamond's publications include: "Observations on the excitation by acetylcholine and by pressure of sensory receptors in the cat's carotid sinus" (*J. Physiol.* 1955, **130**, 513); with J. A. B. Gray & M. Sato, "The site of initiation of impulses in Pacinian corpuscles" (*J. Physiol.* 1956, **133**, 54); and, with A. Howe, "Chemoreceptor activity in the aortic bodies of the cat" (*J. Physiol.* 1956, **134**, 319).

SIR CHARLES LOVATT EVANS is Emeritus Professor of Physiology in the University of London, Consultant to the Ministry of Supply, and Chairman of the Council of the Royal Veterinary College. He was Professor of Physiology in the University of Leeds 1918–19, at St Bartholomew's Medical College 1922–1926, at University College, London, 1926–49, and has been a Fellow of University College since 1918. He worked at the National Institute for Medical Research from 1919 to 1922, and was a member of the Medical Research Council from 1947 to 1950. He has worked in various branches of physiology—for example, on plain muscle, carbohydrate metabolism, the metabolism of the heart, salivary secretion, the actions of various war gases, and latterly on sweat secretion in the horse. Among his publications were the earlier editions of *Recent advances in physiology*, and the *Principles of human physiology* originally written by his teacher, E. H. Starling. Of this he has produced eight editions, the latest (the 12th ed.) being in 1956.

DR W. FELDBERG was Lecturer in Physiology at Berlin University until 1933 when he was forced to leave Germany. He then worked at the National Institute for Medical Research, London, and later at the Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; afterwards he became Reader in Physiology at Cambridge University. Dr Feldberg is now again at the National Institute for Medical Research. His main work has been on the physiology and pharmacology of histamine and acetylcholine. Lately he has been working, in collaboration with Dr S. L. Sherwood, on changes in behaviour produced by injection of drugs into the cerebral ventricles of the unanaesthetized cat.

PROFESSOR R. C. GARRY is Regius Professor of Physiology in the University of Glasgow. He is a graduate of that University, where he was once a Lecturer in Physiology. From 1933 to 1935 he was Head of the Physiology Department in the Rowett Research Institute, Aberdeen. He then held the Chair of Physiology in University, now Queen's, College, Dundee for 12 years. In 1947 he returned to Glasgow. Professor Garry's interest in the physiology of the gastrointestinal tract was due to the influence in rapid succession of Professor Paul Trendelenburg in Freiburg im Breisgau, of Sir Charles Lovatt Evans of University College, London, and of Professor B. A. McSwiney in Leeds. This continues to be his chief pre-occupation. From his sojourn in the Rowett Institute, under the directorship of Lord Boyd Orr, he acquired a cognate interest in nutrition. From 1950 to 1953 he was President of the Nutrition Society of Great Britain.

DR J. A. B. GRAY is Reader in Physiology at University College, London. Previously, he was a member of the Scientific Staff of the Medical Research Council, and worked at the National Institute for Medical Research, London. He has worked mainly on the mechanism of sensory receptors and on the activity of the spinal cord. His publications include: with J. L. Malcolm, "The initiation of nerve impulses by mesenteric Pacinian corpuscles" (*Proc. roy. Soc.* B, 1950, **137**, 96); with M. Sato, "Properties of the receptor potential in Pacinian corpuscles" (*J. Physiol.* 1953, **122**, 610); and, with A. Fernandez de Molina, "Activity in the dorsal spinal grey matter after stimulation of cutaneous nerves" (*J. Physiol.* 1957, **137**, 126).

DR S. M. HILTON has been engaged in full-time physiological research for the past seven years, first in the Physiological Laboratory, Cambridge, and subsequently at the National Institute for Medical Research, London. His main interest has been the regulation of peripheral circulation. Several papers have been published in the *Journal of Physiology*, in the years 1953–56, on the post-contraction hyperaemia of skeletal muscle, and on the mechanism of functional hyperaemia in the submandibular salivary gland. Present work includes a study of vasodilatation in human skin, particularly under conditions in which the sweat glands are activated.

DR O. F. HUTTER is a Lecturer at University College, London. Coming from the Wellcome Physiological Research Laboratories, he entered University College in 1946 to read for a degree in physiology. Afterwards he was Sharpey Scholar under Sir Charles Lovatt Evans and Sir Lindor Brown. Dr Hutter has worked on problems of neuromuscular and ganglionic transmission and has published articles analysing different forms of neuromuscular facilitation. In 1953 he went to the USA as a Rockefeller Fellow to spend two years at Johns Hopkins Hospital in the laboratory of Dr Stephen W. Kuffler. The paper contributed to this number of the Bulletin is a review of work on vagus inhibition done there with Dr W. Trautwein, and of recent experiments on this subject made in London in collaboration with Dr E. J. Harris.

DR G. P. LEWIS commenced whole-time research work at the Welsh National School of Medicine as a Wellcome Research Fellow (1950–52). He studied the relationship between the structure and chemical reactivity and the pharmacological actions of sympathomimetic amines and their antagonists. Since 1953, he has worked at the National Institute for Medical Research, London, at first as a Beit Memorial Fellow for Medical Research (1953–56) and later as a member of the Scientific Staff. Here his main interest has been in the biochemistry of vascular changes occurring under physiological and pathological conditions in glandular organs and in connective tissue. Dr Lewis is at present investigating the formation of pharmacologically active polypeptides

by the action of proteases and proteolytic enzymes on plasma proteins. His main published work has appeared in the *British Journal of Pharmacology and Chemotherapy* and in the *Journal of Physiology*.

PROFESSOR F. C. MACINTOSH is Joseph Morley Drake Professor of Physiology, McGill University, Montreal, and a member of the Medical Advisory Committee of the National Research Council of Canada and of other government committees. From 1937 to 1949 he was a member of the Scientific Staff of the Medical Research Council, during which time he was concerned with the biological standardization of drugs. While working at the National Institute for Medical Research, London, during the Second World War, Professor MacIntosh was interested in personnel research for the Royal Navy. His present research is in the field of chemical transmission, central and peripheral synapses, and in the behaviour of pharmacologically active substances in tissues. Among his publications are: "The distribution of acetylcholine in the peripheral and the central nervous system" (*J. Physiol.* 1941, **99**, 436); with W. D. M. Paton, "The liberation of histamine by certain organic bases" (*J. Physiol.* 1949, **109**, 190). Professor MacIntosh also contributed, with A. S. V. Burgen, "The physiological significance of acetylcholine" (in: Elliott, K. A. C., Page, I. H. & Quastel, J. H., ed. *Neurochemistry*, 1955, p. 311). He was elected a Fellow of the Royal Society in 1954 and a Fellow of the Royal Society of Canada in 1956.

MR J. G. MURRAY is Lecturer in Surgery, University of Aberdeen. Previous appointments included those of Senior Registrar to Professor W. C. Wilson, Department of Surgery, Aberdeen University, 1952; Lecturer in Anatomy under Professor J. Z. Young, F.R.S., University College, London, 1952–54; Clinical Research Fellow of the Medical Research Council under Professor W. D. M. Paton, F.R.S., Department of Pharmacology, Royal College of Surgeons of England, 1954–56. Mr Murray's research work has been concerned with the innervation of viscera and with regeneration in the autonomic and peripheral nervous systems. Among papers published are the following: "Regeneration of non-medullated nerve fibres" (*J. Anat., Lond.* 1954, **88**, 465); with E. Agostini, J. E. Chinnock & M. de Burgh Daly, "Functional and histological studies of the vagus nerve and its branches to the heart, lungs and abdominal viscera in the cat" (*J. Physiol.* 1957, **135**, 182); and, with J. W. Thompson, "The occurrence and function of collateral sprouting in the sympathetic nervous system of the cat" (*J. Physiol.* 1957, **135**, 133).

DR W. L. M. PERRY, O.B.E., M.D., has for ten years been on the staff of the Medical Research Council and, since 1952, has been Director of the Department of Biological Standards at the National Institute for Medical Research, London. He has responsibilities to the World Health Organization for the maintenance of international standard preparations, and to the Ministry of Health for advising on the control of therapeutic substances, including, in recent years, the control of poliomyelitis vaccine. He is a member of the World Health Organization Expert Committee on Biological Standardization, the British Pharmacopoeia Commission, and several Medical Research Council Committees. Dr Perry's principal interests in research have been autonomic function, allergy and anaphylaxis, and the biometrical aspects of the design of experiments; he has published numerous papers in these fields. Before starting research work Dr Perry spent several years in clinical medicine, including two in Nigeria as a medical officer in the Colonial Medical Service.

DR J. W. THOMPSON, now Lecturer in Pharmacology at the Royal College of Surgeons of England, was previously a Price Scholar at the London Hospital, where he subsequently held various house appointments. After serving as a medical officer with the Royal Air Force in Ceylon, he was appointed Research Assistant to the Department of Applied Pharmacology and Therapeutics at University College Hospital, where he worked with Professor (then Dr) W. D. M. Paton, F.R.S. His work has included research on the action of sympathomimetic amines on ganglionic transmission the responses of the smooth muscle of the cat's nictitating membrane both in vivo and in vitro and, latterly, regeneration in the autonomic nervous system.

DR MARTHE VOGT is Reader in Pharmacology, University of Edinburgh. Formerly, she was Head of the Chemistry Department, Kaiser Wilhelm Institut für Hirnforschung, Berlin; Alfred Yarrow Research Fellow of Girton College, Cambridge; and Member of Staff, Pharmaceutical Society of Great Britain. Her work, past and present, is concerned with the action of drugs on the central nervous system, chemical transmitters at synapses, distribution of pharmacologically active substances in the brain, and with the physiology of the adrenal cortex and of the adrenal medulla. Her publications have appeared mainly in the *Archiv für experimentelle Pathologie und Pharmakologie*, the *Journal of Physiology* and the *British Journal of Pharmacology*. Dr Vogt was elected a Fellow of the Royal Society in 1952.

Book Reviews

Modern Trends in Neurology (Second Series)

Edited by *Denis Williams*. London: Butterworth & Co. (Publishers) Ltd., 1957. xi + 350 pages + 24 pages index; 80 figures. 25 × 17 cm. £3 12s. 6d.

The impact on clinical medicine of the basic sciences, which their developing technology in the last twenty years has made possible, came rather later to neurology than to other specialties. This second volume in the Modern Trends series shows a welcome change from most special texts devoted to neurological subjects. The emphasis in the 24 essays is not upon the finer points of nosological classification, and there is little descriptive symptomatology—knowledge of which is assumed in the reader. The editor, Dr Denis Williams, in choosing a bias towards the basic sciences upon which the future development of the subject depends, has thrown his net widely to bring in contributors each of whom has had special experience of the subject of his essay. Nearly half the chapters are concerned with anatomy, biochemistry, physiology or pathology, but there is, surprisingly, very little electrophysiology.

After an essay by P. W. Nathan largely devoted to the thalamus, which is well illustrated by schematic drawings, J. N. Cumings provides an interesting review of metabolic disorders affecting the cerebrum, including hepatolenticular degeneration, hepatic coma, and the lipidoses. Chapters by W. H. Sweet and J. Foley are devoted to the physiology of the cerebrospinal fluid, the second of which is a comprehensive but very clear and well-documented account of the mechanisms underlying intracranial hypertension. A high point in this book is reached in the two chapters by C. E. Lumsden. In the first he gives an account of present knowledge of the enzymes associated with medullated nervous tissue, the possible role of porphyrins and the problem of trace substances in multiple sclerosis. The second chapter takes the question of myelination and demyelination further in an account of the molecular structure of myelin as revealed by electron microscopy, and of Géren's work on the development of the myelin sheath. The ultrastructural arrangement in which the lipid layers alternate with protein layers would seem to give the myelin sheath its extremely high insulating capacity.

A series of essays bring together new knowledge of vascular disorders, including a review by Sir Charles Symonds of internal carotid occlusion and its relief by surgery. The natural history of this condition is not yet known, nor how many patients suffer it without major symptoms. The prospect of surgical relief has given a new impetus to early diagnosis. This book indeed reflects the greatly increased role of the surgeon in the treatment of neurological disease, seven of the chapters being contributed by surgeons. These deal with such different topics as the pattern of mid-brain deformity in expanding intracranial lesions, by R. T. Johnson; cervical spondylosis, by Valentine Logue; and psychosurgical procedures, by J. Lawrence Pool. Chapters of special clinical interest are concerned with acute viral encephalomyelitis, by Helen Dimsdale; sarcoidosis, by M. Jefferson; and diabetic amyotrophy, by H. Garland. A useful brief review of the present position of clinical electromyography is provided by R. W. Gilliatt. It does not seem likely, however, that, as he suggests, this technique will become widely used by clinical neurologists themselves. Epilepsy, upon which there has been so much recently written, is treated conservatively in this book, the electrophysiological aspects being omitted. Denis Williams surveys the role of the temporal lobe from the clinical point of view, A. Meyer gives an account of hippocampal lesions found in temporal lobe epileptics, and there is an appraisal by Donald B. Tower of the status of medical treatment of seizures, which includes a discussion of the possible mechanisms of anticonvulsant drug action.

This book assumes a high level of knowledge in the reader. Each contributor surveys current knowledge in his chosen field and, because each has been involved in that field, the chapters are for the most part lively, stimulating and forward-looking. There are many who, although not clinical neurologists themselves, have an interest staked in the now expanding field of the science of brain functions. To all these this book has something to offer, and it can be recommended as a source of reference and as an up-to-date statement on many topics of contemporary interest. The book has many excellent illustrations and is beautifully produced.

Denis Hill

Functions of Autonomic Transmitters

J. Harold Burn. Baltimore: Williams & Wilkins Company, 1956. (The Abraham Flexner Lectures.) ix + 228 pages; 43 figures. 21 × 14 cm. £2

This book is a record of the Abraham Flexner Lectures given in Vanderbilt University, Tennessee, by Professor Burn in 1956. He first pays tribute to Flexner's interest in medical education by discussing the differences between the American and British approaches to the instruction of medical students. He then reviews the changes in the relation of theory to experiment in medical science which have occurred since the time of Harvey.

The main body of the book, however, gathers together in related sequences some of the pharmacological studies of Professor Burn and his collaborators. First, the evidence from cardiac studies which led to the hypothesis that the endogenous formation of acetylcholine might be responsible for the rhythmical activity of organs is considered. He then shows how the responses of the cilia in the nerve-free gill plates of *Mytilus edulis* were used to test the wider application of this theory. Next, some of the actions of noradrenaline are examined in the light of ideas and experiments of the Oxford School which have added considerably to our understanding of the role of this substance in the body.

The author's interest in common properties of different classes of drugs is unfolded in chapter V. He illustrates this by pointing out that, although procaine, atropine, papaverine and pethidine have widely different pharmacological actions, they have one property in common, namely that of producing a quinidine-like action on the isolated auricles of the rabbit, and he seeks an explanation of this and similar phenomena.

During the analysis of the alterations in sensitivity to drugs following chronic denervation, evidence is marshalled to show that, after the sympathetic supply to an organ is cut, the decline in amine oxidase cannot be held to account for all the changes observed in drug responses. The unexpected finding that, in the denervated dilator pupillae muscle, the amine oxidase content remains normal, whereas the non-specific cholinesterase falls as supersensitivity to adrenaline develops, is but an example of many seemingly unconnected facts which are subjected to critical assessment.

The final chapter has the impelling title of " Our national drugs—alcohol and nicotine " and, in it, the author amply achieves his object to present the facts impartially, so that persons other than pharmacologists can form their own conclusions. It terminates with a nostalgic plea that the churchwarden pipe with its 14-inch stem should commend itself to those who wish to reduce the amount of tobacco tar reaching their lungs.

The text of this volume preserves admirably the pleasant direct style of discussion of scientific topics for which Professor Burn is so well known and which contributes greatly to the pleasure of reading the book. The reviewer found that his interest was firmly gripped at the beginning and effortlessly maintained to the final page. All medical scientists will be most grateful to Professor J. H. Burn for this scholarly exposition of his approach to research which has proved so stimulating to English-speaking pharmacologists.

J. P. Quilliam

The Neurohypophysis

Edited by *H. Heller.* London: Butterworths Scientific Publications, 1957. (Proceedings of the Eighth Symposium of the Colston Research Society held in the University of Bristol April 9th–April 12th, 1956.) xv + 275 pages; plates; figures. 25 × 19 cm. £2 10s.

The post-war years have seen rapid advances in the subject of this volume. Views on the site of elaboration of neurohypophysial hormones have undergone a radical change. The polypeptide structure of the two hormonal principles has been ascertained and synthetic hormones prepared. Oxytocin has belatedly graduated to full hormonal status. These and a variety of other gains are discussed by a formidable array of authors in this excellently produced book. Containing the papers presented at a symposium of the Colston Research Society, complete with subsequent discussion, the work represents the first occasion on which the neurohypophysis has been subjected to such concentrated scrutiny from so many angles. The wealth of new material not yet to be found in the text-books underlines the timeliness of publication, and the efficient coverage of recent researches reflects credit on the selection of contributors.

Admirable surveys of the growing field of neurosecretion are provided by W. Bargmann and B. Hanström, with particular reference to pituitary relationships. R. Acher & C. Fromageot discuss the separation of oxytocin and vasopressin polypeptides from posterior pituitary protein and give evidence of differing pituitary ratios of the two polypeptides in growth, parturition and lactation. Recent observations on the vasopressin : oxytocin ratios in various hypothalamic regions are detailed in a critical review on storage and release of neurohypophysial hormones, by H. B. van Dyke, K. Adamsons & S. L. Engel. H. Croxatto considers the formation and properties of posterior pituitary-like polypeptides extracted from serum. The sites of inactivation of circulating neurohypophysial hormones are analysed by H. Heller, and R. L. Noble discusses the limitations of urine antidiuretic assays.

A challenging contribution by M. Ginsburg & L. M. Brown suggests that, contrary to accepted views, vasopressin may have a physiological role in cardiovascular homeostasis. Unrepentant, they then proceed to throw doubt on the existence of a mechanism in the rat for releasing antidiuretic hormone when hypertonic saline is infused into the internal carotid artery. More novelties are forthcoming from H. Wirz who assembles data from his elegant renal micropuncture studies in order to construct a " hair-pin countercurrent " theory of antidiuretic hormone action which involves the reabsorption of water from the collecting tubules. Amphibian water-balance mechanisms include additionally neurohypophysial effects on skin and bladder; these phenomena are discussed by W. H. Sawyer. F. P. Brooks & M. Pickford are disposed to implicate both vasopressin and oxytocin in the control of sodium and potassium excretion under certain conditions in dogs. B. Andersson describes the production of polydipsia, antidiuresis and milk ejection in conscious goats by stimulation of the hypothalamus.

The difficult terrain of adrenocortical-neurohypophysial interrelationships is explored in presentations by I. Chester Jones and R. Gaunt, C. W. Lloyd & J. J. Chart. Oxytocin comes eventually into prominence in a useful discussion on uterine function by R. J. Fitzpatrick and in a review on the role of the neurohypophysis in lactation by A. T. Cowie & S. J. Folley, which includes the results of new experiments supporting the belief that oxytocin stimulates anterior pituitary secretion of prolactin. J. M. Walker reports blood-assay techniques for oxytocin and vasopressin which have been used to study the releasing effect of various drugs on the two hormones.

Not the least rewarding part of the book is the introductory survey by Sir Henry Dale of the milestones of discovery and concept leading to the present-day knowledge. His frequent contributions to the discussions, moreover, provide a fascinating critical commentary on modern ideas of neurohypophysial function.

B. A. Cross

Physiology of the Nervous System

E. Geoffrey Walsh, with chapters on somatic sensibility and the applied physiology of pain by *John Marshall.* London: Longmans, Green & Co., 1957. xvi + 563 pages; 6 plates + figures. 23 × 15 cm. £2 10s.

This is a scholarly book—written by a scholar and appealing primarily to scholars, the publishers' blurb on the dust-cover notwithstanding. Readers who hope to find within its covers a compressed encyclopaedia of our knowledge of the physiology of the nervous system will be disappointed. There is scant reference to the basic work of Eccles on the motor neurone, and the various current ideas on transmitter substances are scarcely mentioned, let alone discussed. It would be all too easy to list other examples of such omissions, but such absences are more than compensated for by the clinical and historical approach. Every chapter contains a wealth of quotation from authorities of all ages. While the citations of current literature receive rather scant discussion, the quotations inserted for their historical significance are very full and it is a frequent delight to discover how well expressed were the findings and ideas of an older generation.

The arrangement of the book has some very commendable points. The spinal cord reflexes are dealt with mainly in a chapter headed " Posture . . . ", and the integration that such an arrangement provides makes this one of the most readable chapters in the book. Similarly, no separate section is devoted to the autonomic nervous

system, but rather its role is emphasized wherever it plays a part in an integrated pattern of responses. To readers of this Bulletin, this may be disappointing, but it should act as a salutary reminder that the nervous system works as a whole, and that the findings of analytical studies must eventually be integrated with the activity of the complete organism.

It is a little alarming to feel, however, that the author considers that this book provides the physiological basis that the clinician needs. If the more specialized physiology of the motor neurone is considered out of place in a book such as this, might not better use have been made of the available space by giving less to citations from current literature and more to illustrating basic principles of the behaviour of neurones in various situations in the central nervous system? Nonetheless, the book is very readable and, if only on account of the 63 pages of references, it should appeal to a large public.

J. Laurence Malcolm

The Life of Mammals

J. Z. Young. Oxford: Clarendon Press (London: Oxford University Press), 1957. xv + 820 pages; 338 figures. 24 × 16 cm. £4 4s.

In his preface to this remarkable volume, Professor Young indicates that he is attempting a compromise between the conventional presentation of the established facts of mammalian structure and function, and a " better " method in which many parts of biology " can be illuminated by use of a new language that considers the body as a self-controlling machine ". This new language is one founded on the terminology of modern communication theory. In the first chapter, on the control of living systems, a succinct account is given of the author's general attitude, together with a discussion of biological method; it includes a summary of the characteristics of living organization. The beginner is recommended by Professor Young to read this chapter before he passes on to the remainder. Paradoxically, however, this advice should probably have been given to those initiated into biology and who speak in more old-fashioned languages. It will be these, rather than the tyros, who will find the difficulties in the understanding, and in the acceptance, of all the new idioms. A sympathetic reading of the general statement of the author's attitude and approach—and it deserves and demands close attention—will greatly increase the understanding of the book as a whole, and will, indeed, be found to be an exciting intellectual experience. None will agree with all of the interpretation, but there will surely be few who will find this attempt at a new biological synthesis other than exciting and exhilarating.

As is to be expected from Professor Young's research interests and contributions, the nervous system receives extensive and authoritative treatment in the general text, accounting, as it does, for about a quarter of the volume. The significance of the nervous system in mammalian life and its central importance in the development of ideas on control mechanism more than justify this preferential treatment. Anyone interested in any aspect of nervous activity will find Professor Young's account of nervous tissue, and of the central and peripheral nervous system, most interesting. For the general biologist, however, it will be the extension of the concept of control in living systems to tissues and organs other than the nervous and endocrine systems which will perhaps be more arresting because of greater novelty.

There is so much factual detail and fresh material in the volume that a review that would do justice to it would have to be both extensive and by a number of authorities. The author himself confesses that he has not been able, either exactly or fully, to apply the technique of information theory to all the problems posed by mammalian organization; he has, however, demonstrated most clearly how interesting its application makes the consideration of the structure and functions of many parts of the body.

The volume is beautifully printed and contains a large number of attractive illustrations.

J. D. Boyd

Mathematics and Statistics for Use in Pharmacy, Biology and Chemistry

L. Saunders & R. Fleming. London: Pharmaceutical Press, 1957. x + 257 pages; figures. 23 × 14 cm. £1 7s. 6d.

This is a remarkable book which attempts the impossible and very nearly pulls it off. Mathematics is, primarily, a state of mind, which, if not inborn, can be moulded and developed by adequate teaching; a useful knowledge of mathematics cannot be provided by a short book. To those who know nothing of the calculus, 30 odd pages of text, however admirably written, cannot give more than a superficial and transient impression of the techniques of differentiation and integration. What seems to the authors, who have long acquired the necessary attitude of mind, a very simple exposition, will appear to the uninitiated an overwhelmingly difficult condensation.

The book must, therefore, be said to fail, but to fail gloriously, in its avowed aim, namely, to " . . . offer a short course in mathematics and statistics which assumes very little knowledge of either topic", to students who have passed the matriculation examination but who " . . . know nothing about calculus ". Nevertheless, it has a very real value to another group altogether, namely to those who have studied the subject-matter before—perhaps even a very long time before: to them it will not be new and difficult; it will be an admirable summary of facts and methods long relegated to limbo. The authors combine clarity and brevity of style, which implies clear thinking, and cover their wide field accurately and in logical order. To this limited public, therefore, this book will be a valuable addition to the bookshelf.

W. L. M. Perry

Radioactive Materials and Stable Isotopes: Catalogue No. 4

Isotope Division, Atomic Energy Research Establishment. Harwell: 1957. 221 pages; figures. 24 × 16 cm. (Obtainable on application to Isotope Division, A.E.R.E., Harwell, Didcot, Berkshire.)

Isotope tracer techniques are now of established value in practically every branch of medical science, and are being increasingly used for diagnostic purposes. The rapid progress which has been made in the application of these new techniques owes much to the services provided by the Isotope Division at Harwell. These services are summarized in the present volume, but not the least of these is the provision of the catalogue itself, since it is a mine of information of considerable practical value to anyone who uses, or who contemplates using, isotope techniques.

The greater part of the catalogue is devoted to an alphabetically arranged list of some 140 isotopes prepared in the pile by neutron irradiation of the appropriate target materials, or which are extracted from fission products. In each case a summary of the radiation characteristics of the isotope is given, together with information on the target materials used, radioactive impurities produced (if any) and the specific activities available. Stable isotope preparations are listed under a separate section. A table of definitions covers the specialized technical terms used in these sections.

There is an excellent section which deals with health hazards arising from the use of radioactive materials, and indicates the practical measures that may be taken to control them. In many countries the use of radioactive isotopes is subject to varying degrees of control by official bodies, and a list of the appropriate authorities for those countries is included.

Apart from the supply of radioactive and stable isotopes, other services include the preparation of radioactive standards, the isotope advisory service, irradiation facilities for activation analysis, and the training courses provided by the Isotope School at Harwell. Labelled compounds, separated isotopes, applicators for radiotherapy and naturally radioactive materials are not included in the A.E.R.E. catalogue, since they are covered by a catalogue issued by the Radiochemical Centre, Amersham, Buckinghamshire.

N. V.

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British Medical Bulletin

Autonomic Nervous System

INTRODUCTION	*Sir Henry Dale*
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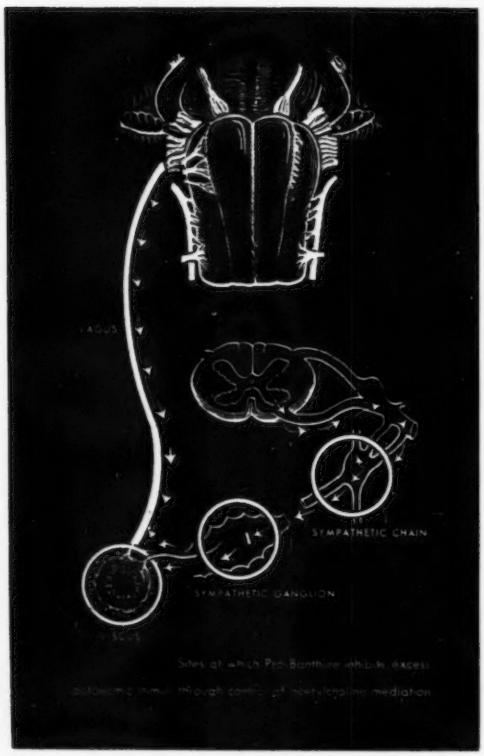

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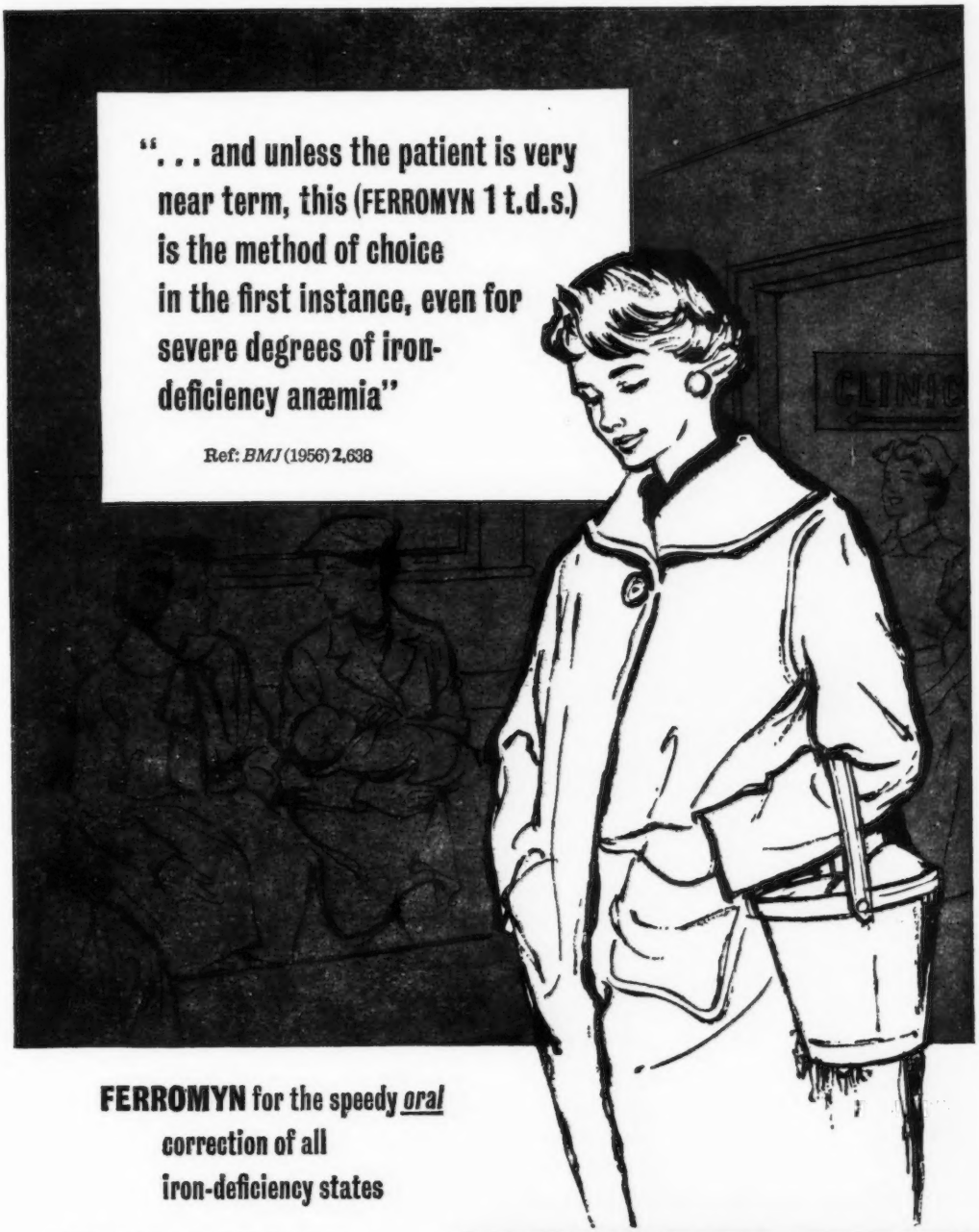
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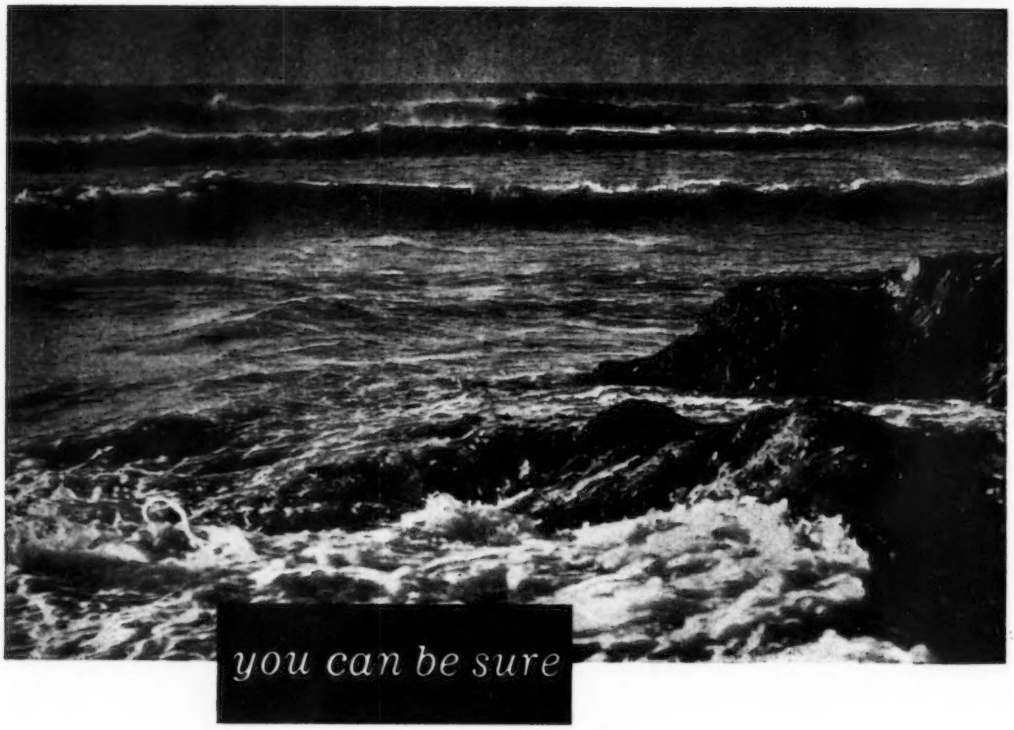

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